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IDENTIFICATION OF TYPES AND PRIMARY SITES OF METASTATIC TUMORS FROM EXFOLIATED CELLS IN SEROUS FLUIDS*

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THE PROBLEM

It is well established that cells from malignant tumors can be readily recognized as such in smears or cell-block preparations of serous fluids; in only about 10 per cent of the examinations will there be any doubt as to the validity of the diagnosis.[1-7] In this article we are dealing with a somewhat different and more special problem: Can the primary site and type of the tumor from which cells have exfoliated also be deduced with any degree of accuracy when the diagnosis is based upon their cytologic characteristics? It is often difficult or impossible to determine these points by examining stained sections of a metastasis in a lymph node or other tissue. How much more difficult, then, will it be to make such a diagnosis on the sole basis of appearance of cells, or clusters of cells suspended in a serous fluid? Offhand, one would be very doubtful as to the accuracy of the results of such an examination. Consequently the work reported in this paper was undertaken in an attempt to confirm or to dispel such doubt.

Earlier investigations on the identification of types of pulmonary cancer in smears of sputum and bronchial aspirates[8] yielded sufficiently encouraging results to afford considerable stimulation to the investigation of serous fluids.

The results of the present experiment are interesting since they show that, while the accuracy of diagnosis of types and sites made on a series of smears taken consecutively as they come into the laboratory is admittedly poor, it is rather the reverse in so far as the more common tumors comprised in the series are concerned. Five or six kinds

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of malignant tumors constitute 80 per cent of the lot and the diagnosis of primary site and type in this group averages about 68 per cent correct, but the remaining 20 per cent show an accuracy of diagnosis that averages only 18 per cent. While an average of 68 per cent is worth while considering, the over-all average of 45 per cent would discourage further investigation if one did not know why it is so low. It will be shown, however, that there is an encouraging side to the figures; the diagnosis of mammary, bronchogenic, and gastric carcinoma, and of the group commonly known as "the lymphomas," is 70 per cent correct, and these tumors constitute more than half the series (64 per cent). The only other tumor largely represented is ovarian carcinoma (16.4 per cent of series), in which the degree of accuracy is only 58 per cent, too low to be very encouraging. Thus, if it be known which types can be diagnosed with satisfactory accuracy and which cannot, something can be accomplished in attacking the problem.

Of course, there is no hope of taking preventive or curative measures when a patient has generalized metastasis so severe as to flood the serous cavities with malignant neoplastic cells; the outlook is hopeless. Nevertheless, there are inflammatory and mechanical conditions that provoke serous exudates and it often happens that these are mistaken for the manifestations of the presence of metastatic tumor. It is distinctly worth while to rule out the latter and equally so to be in a position to state that the process is frankly inflammatory or mechanical. While this paper is possibly more interesting from an academic than from a practical standpoint, it will bring out definite conclusions that are offered at face value.

Material

The material comprised smears from serous fluids from the pleural, peritoneal, and pericardial cavities of 219 patients. In order to set up this series it was necessary to tap a series of 2,027 reports, as only those could be utilized in which a positive diagnosis of cancer had been made on the smears and, furthermore, the primary site had been established on the basis of necropsy, biopsy, or a definite history pointing to the recurrence of a previously diagnosed malignant tumor.

A preliminary diagnostic run was made on the first 100 of the 219 specimens and, after checking the results for correctness, the smears were carefully reviewed and those in which the diagnosis had been erroneous were studied with particular care to determine whether the average accuracy of diagnosis could not be improved through the discovery of additional diagnostic criteria. It was found that the results of the second run or review of the material yielded approximately the

same number of successes and failures in accurate diagnosis. The remaining 119 smears were next examined and diagnosed and the results pooled with those of the preliminary survey. A summary of the series will be found in Table I.

Report on the Diagnosis of the More Common Tumors

It is best to discuss the results in detail where the more common tumors are concerned and these will be taken up in the order of their frequency of occurrence in the series.

While studying the smears the following points were always kept in mind: (a) the abundance or scarcity of tumor cells in the microscopic field; (b) the size of the cells, their shape, the appearance of their cytoplasm, and the distinctness of their outline; (c) the same factors were observed for the nuclei, and multinucleation was noted when it occurred; (d) the presence of "proliferation spheres" (to be described later) and of abortive structures like tubules was noted also; (e) the presence and type of other, non-neoplastic elements in the smears.

Mammary Carcinoma

Number of cases: 57. Percentage of series: 26.0. Correctly diagnosed: 40 (70.0 per cent). Incorrectly diagnosed: 15 (27.0 per cent). No diagnosis of type possible: 2 (3.0 per cent). Males: 1 (2.0 per cent). Females: 56 (98.0 per cent). Pleural exudates: 51 (89.5 per cent). Pericardial: 1 (1.7 per cent). Peritoneal: 5 (8.8 per cent).

Cytologic Characteristics. The cells of mammary carcinoma are usually not numerous, and are large or medium-sized, spheroidal, and distinctly outlined. Their cytoplasm is either pale and vacuolated, or homogeneous and hyaline, the former type predominating slightly. In all cases the nuclear outline is distinct, the nuclei usually being large and ellipsoidal, less often spheroidal. Their karyoplasm is structured, showing a pattern of karyosomes and threads. Multinucleation is only seldom noted. Very occasionally there may be a suggestion of a double cell-contour. The cells may be arranged concentrically into characteristic spheres which may be called proliferation spheres, as they seem to represent a multiplication of free-lying cells in the fluid. This is a very useful diagnostic feature as it is noted only in mammary and ovarian carcinomas; unfortunately, it is present in only one third to one half of the smears. Otherwise, the arrangement of cells takes the form of disorderly clumps or abortive tubular structures. Smears comprising numbers of discrete cells without any tendency to cluster are a rarity. The non-neoplastic cellular elements in these smears are usually lymphocytes and histiocytes.

TABLE I

Data on Types of Tumor in the Survey

Tumor	No. in series	Percentages				Fluids					
						Pleural		Abdominal		Pericardial	
		In series	Correct diagnosis	Males	Females	No.	%	No.	%	No.	%
Mammary carcinoma	57	26.0	70.0	2.0	98.0	51	89.5	5	8.8	1	1.7
Bronchogenic carcinoma	49	22.4	77.0	77.0	23.0	49	100.0	0	0.0	0	0.0
Ovarian carcinoma	36	16.4	58.0	0.0	100.0	10	27.8	26	72.2	0	0.0
Gastric carcinoma	17	7.8	71.0	47.0	53.0	4	23.5	13	76.5	0	0.0
Intestinal carcinoma	12	5.5	50.0	42.0	58.0	3	25.0	9	75.0	0	0.0
Pancreatic carcinoma	10	4.6	0.0	50.0	50.0	1	10.0	9	90.0	0	0.0
Carcinoma of uterus and cervix	7	3.2	0.0	0.0	100.0	5	71.4	2	28.6	0	0.0
"Lymphoma"	18	8.2	66.7	55.6	44.4	13	72.2	5	27.8	0	0.0
Other tumors	13	5.9	21.3	Irrelevant		8	61.5	5	38.5	0	0.0
Totals	219	100.0	46.0	Average		144	65.7	74	33.8	1	0.5

If the percentages of correct diagnosis be averaged only for the 178 tumors that occurred to the extent of over 7.7 per cent of the series, the low average of 46.0 per cent can be raised to approximately 68 per cent. The text will explain why such a step would not be unreasonable.

Bronchogenic Carcinoma

Number of cases: 49. Percentage of series: 22.4. Correctly diagnosed: 38 (77.0 per cent). Incorrectly diagnosed: 10 (21.0 per cent). No diagnosis of type possible: 1 (2.0 per cent). Males: 38 (77.0 per cent). Females: 11 (23.0 per cent). All of these exudates were of pleural origin.

Cytologic Characteristics. The cells of bronchogenic carcinoma are usually not numerous, and are large and spheroidal, with distinct outline. Their cytoplasm may be pale and vacuolated (in adenocarcinomas) or homogeneous and sometimes slightly keratinized and orangeophile (in the more numerous epidermoid variety). The nuclei are slightly more often large than small, or medium-sized, again depending upon the type of tumor. Sausage-shaped nuclei sometimes are noted, but those with an elliptic shape are more common. Their outline is always sharp and distinct, and their karyoplasm structured in about half the cases. Multinucleation is noted in about 43 per cent of the smears and this is a distinctive feature, as it is seldom noted outside of the bronchogenic group (occasional in ovarian and mammary carcinoma). The cells are usually arranged in *small* groups of from three to eight elements. Proliferation spheres were noted in only three of the 49 cases (6 per cent). Pus is more commonly present in the smears of bronchogenic carcinoma than it is in any of the other groups examined. This is probably attributable to secondary infection from broken-down, cancerous lung.

Ovarian Carcinoma

Number of cases: 36. Percentage of series: 16.4. Correctly diagnosed: 21 (58.0 per cent). Incorrectly diagnosed: 14 (40.0 per cent). No diagnosis of type possible: 1 (2.0 per cent). Pleural exudates: 10 (27.8 per cent). Peritoneal: 26 (72.2 per cent).

Cytologic Characteristics. The cells are usually numerous, large or medium-sized in about equal proportions, and usually spheroidal. Their outlines are distinct when they lie discretely separated in the smear, but, when they are clustered, outlines are so indistinct as to give the clumps a syncytial appearance. In 19 per cent of the smears there was an apparently double contour of the cell-membrane, an outer and distinct ring and an inner one that was too vague to constitute a true double contour like that of blastomyces. However, there is a conspicuously clear border zone that gives an impression of a double contour. The cytoplasm is usually clear, pale, and conspicuously vacuolated; rarely dense with small vacuoles, or variable. The nuclei are usually medium-sized; they are ellipsoidal in three fourths

of the cases and sharply outlined. More than one third show a structured karyoplasm, while one fourth are laked or diffuse. The rest of them are densely staining, almost to the point of pyknosis. Proliferation spheres are very characteristic when present, but unfortunately they occur in only 36 per cent of the smears. They are less concentrically arranged than are those of mammary carcinoma, but they are distinctly outlined by a membrane of peripherally situated cells. Disorderly clusters of variable size are often noted. One smear showed cells grouped into short, abortive, duct-like arrangements. Non-neoplastic elements are chiefly lymphocytes and histiocytes.

Gastric Carcinoma

Number of cases: 17. Percentage of series: 7.8. Correctly diagnosed: 12 (71.0 per cent). Incorrectly diagnosed: 4 (23.0 per cent). No diagnosis of type possible: 1 (6.0 per cent). Males: 8 (47.0 per cent). Females: 9 (53.0 per cent). Pleural exudates: 4 (23.5 per cent). Peritoneal: 13 (76.5 per cent).

Cytologic Characteristics. The cells of gastric carcinoma are usually very sparsely distributed and hard to find. They are small and spheroidal in about one third of the smears and elliptic in 20 per cent. The rest may be angulated, elongated, or sausage-shaped. Slender fusiform cells appear often enough to be very characteristic when present. Cellular outline is always distinct and the cytoplasm is pale and vacuolated in 70 per cent, granular in 20 per cent, in the rest, clear and watery. The nuclei are either large or medium-sized in proportion to the cell, their outline being distinct and ovoid rather than elliptic. Their karyoplasm is structured and rather dense. Cell clusters are small and may take the form of abortive tubules, but they are less numerous than in smears of other tumors. Lymphocytes and histiocytes are the usual non-neoplastic elements.

Lymphoid Tumors

Number of cases: 18 (including 3 of Hodgkin's disease). Percentage of series: 8.2. Correctly diagnosed: 12 (66.7 per cent). Incorrectly diagnosed: 5 (27.8 per cent). No diagnosis of type possible: 1 (5.5 per cent). All of the Hodgkin's disease cases were correctly diagnosed as such. Males: 10 (55.6 per cent). Females: 8 (44.4 per cent).

Cytologic Characteristics. The cells of the lymphoid tumors are usually extremely numerous. They vary in size with the type of lymphoma present (small lymphocytes, large lymphocytes, and, in Hodgkin's disease, large pale cells with vesicular nuclei reminiscent of histiocytes). The variability in the cells in the smears at once arouses

suspicion of a lymphoma, rather than of a lymphocytic inflammatory exudate, in which they would be small and uniform. They present manifest abnormalities in size, shape, and density. It is important that they are evenly distributed throughout the smear as discrete elements and that they do not cluster. They are distinctly more numerous than are the cells of carcinomas. The presence of eosinophilic leukocytes in the exudates of Hodgkin's disease is a very useful and generally available diagnostic feature. Large "Hodgkin's sarcoma cells" may be occasionally noted; however, typical Reed-Sternberg cells are difficult to recognize in smears of serous fluids, although they can be readily found and identified in contact smears from granulomatous lymph nodes.

Other Tumors

Other tumors in the series were not represented sufficiently often to make detailed analysis of their cellular characteristics either feasible or accurate. There were twelve intestinal carcinomas, half of which were correctly diagnosed. Their cells were large, the cytoplasm pale

TABLE II

Data on "Other Tumors" in Last Category of Table I

Tumor	No.	Percentage	Diagnosis	Fluid		Sex	
				Pleural	Abdominal	Male	Female
Rhabdomyosarcoma	2	0.9	None possible	1	1	1	1
Malignant melanoma	2	0.9	Incorrect	2	0	1	1
Mesothelioma	1	0.5	Incorrect	0	1	1	0
Hepatic carcinoma	1	0.5	Incorrect	0	1	0	1
Carcinoma of gallbladder	1	0.5	Incorrect	0	1	0	1
Carcinoma of tongue and esophagus	1	0.5	Correct	1	0	1	0
Transitional cell carcinoma of bladder	1	0.5	Incorrect	1	0	1	0
Carcinoma of buccal mucosa	1	0.5	Correct	1	0	1	0
Wilms' tumor	1	0.5	Incorrect	0	1	0	1
Liposarcoma	1	0.5	Incorrect	1	0	1	0
Osteosarcoma	1	0.5	Incorrect	1	0	1	0
Totals	13	5.9		8	5	8	5

The two "correct" diagnoses were correct only in so far as they recognized highly keratinized epidermoid carcinoma, the primary site of which was conjectural.

and vacuolated, and their nuclei vesicular. The arrangement of the cells was irregular, but sometimes suggested the attempted formation of tiny papillae. This is, however, a mere list of equivocal features and the resulting 50 per cent in accuracy is no better than a random guess.

Nothing was noted from pancreatic, uterine, or intestinal tumors

TABLE III

Summary of Cellular Characteristics in Five Groups of Malignant Tumors

Tumor	Cells			Cytoplasm	Double contour	Nuclei			Multinucleation	Proliferation spheres	Tubules
	Number	Size	Shape			Size	Shape	Karyoplasm			
Mammary carcinoma	Few	L	Sphr.	Var.	No	L	Var.	Struc.	Occasional	Yes	Occasional
Bronchogenic carcinoma	Usually few	L	Sphr.	Var.	No	L	Ellip.	Struc.	Yes	No	No
Ovarian carcinoma	Numerous	L-M	Sphr.	P & V	Yes	M	Ellip.	Var.	Occasional	Yes	Seldom
Gastric carcinoma	Few	Small	Var.	P & V	No	L	Oval	Struc.	No	No	Yes
Lymphoid	Very numerous	Var.	Sphr.	Var.	No	L	Sphr.	Struc.	No	No	No

L = large, M = medium, Var. = variable, Sphr. = spheroidal, Ellip. = ellipsoidal, Struc. = structured (showing a pattern of karyosomes and filaments), P & V = pale and vacuolated.

that could be considered to be even reasonably diagnostic. As for the two myosarcomas, their cells were completely primitive and in no way suggested myoblasts. The cells in the two instances of malignant melanoma so seldom showed any intracellular pigment that the diagnosis was missed. One smear did present prominently pigmented cells that could be recognized as probable melanoblasts, but this was a single smear from a number of negative examples. While it is possible that mesothelioma might be diagnosed on the basis of a serrated outline to its cells (produced by a peripheral ring of small vacuoles), this feature was absent in the one case in this series and the diagnosis was missed.

Sundry Observations on the Survey

Fluids. There were 144 pleural, 74 peritoneal, and one pericardial fluid in the collection of smears; this represents percentages of 65.7, 33.8, and 0.5 respectively. The reason for the preponderance of pleural exudates can best be explained on the basis of the site of the majority of tumors in the set. Mammary and bronchogenic carcinomas together comprised 48.4 per cent of the cases and several of the other types of carcinoma exhibited a surprising predilection for intrathoracic metastasis; for instance, 72.2 per cent of the lymphomas and 27.8 per cent of the ovarian carcinomas were submitted as smears of pleural effusions.

Importance of Clinical Data. From what has been said, it might appear that the diagnosis of types and sites of tumors was based purely on objective data. While the study was purposely kept as objective as possible, it would be a mistake to observe this objectivity while making routine examinations in the laboratory. Any diagnostician is entitled to know all the facts about his case and a knowledge of the clinical history of each is imperative. The source of the fluid, the sex of the patient, and his past clinical history will make the reading of the smears far easier. To illustrate: let us postulate that a smear of fluid is examined and diagnosed objectively; the cellular characteristics of the specimen will give certain clues which will be sufficient to enable the cytologist to make a fairly positive diagnosis. But if the data submitted with the specimen indicate that the fluid is pleural, that the patient is an elderly woman whose left breast was removed 5 years earlier for carcinoma, the diagnosis becomes well rounded out. Should the results of cytologic examination and the clinical history be at serious odds, it will behoove the clinician to search for a possible second tumor in the case, for the occurrence of multiple primary malignant tumors of different sorts is not uncommon.

Summary and Conclusions

It is possible to diagnose the type and source of malignant tumor cells in serous effusions with an over-all accuracy of about 50 per cent. This figure may be greatly improved by reckoning only those tumors which are most commonly found in a series of smears of such effusions, such as mammary, bronchogenic, and gastric carcinoma and "lymphomas" of various types.

The degree of accuracy is influenced largely unfavorably by the fact that ovarian carcinomas (which rate as "numerous" in such a series) cannot be more accurately recognized. They often invade the pleura (which is misleading) and they may be quite equivocal in regard to their cytologic features. Tumors less commonly found in smears of serous fluids, such as sarcomas, mesotheliomas, and melanomas, are fortunately so few in number that mistakes in diagnosis need not discourage us. If the majority of tumors can be diagnosed with a degree of from 65 to 76 per cent of accuracy, the method is surely worthy of consideration and of further study.

All photomicrographs were taken by Miss Suzanne Voorhies of the Department Staff.

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LEGENDS FOR FIGURES

Figs. 1 to 7 are examples of cells from mammary carcinoma.

FIG. 1. Single "proliferation sphere," typical of mammary carcinoma. × 600.

FIG. 2. A pair of proliferation spheres, showing the concentric arrangement of cells. × 600.

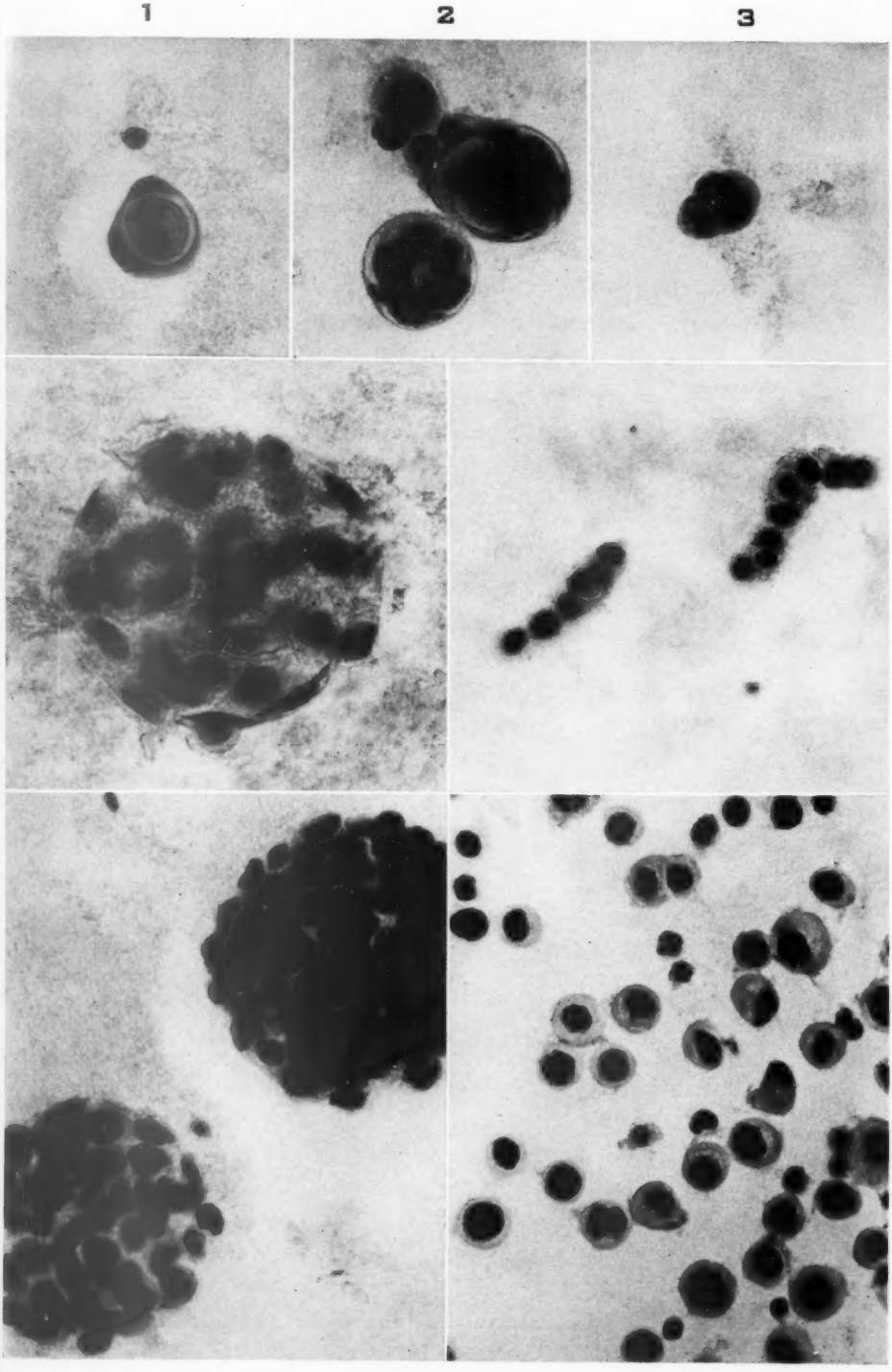
FIG. 3. Proliferation sphere in early stage of formation. × 600.

FIG. 4. A larger and differently constituted proliferation sphere. × 600.

FIG. 5. Two chains of cells suggesting the scirrhous form of carcinoma. × 600.

FIG. 6. Two large, simple clusters similar to those often seen in ovarian carcinoma. × 600.

FIG. 7. Unusually copious exfoliation, one cell binucleate. × 600.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Figs. 8 to 15 are examples of cells from bronchogenic carcinoma.

FIG. 8. Heavy exfoliation of an epidermoid bronchogenic carcinoma. × 600.

FIG. 9. Single keratinized cell surrounded by pus cells, epidermoid type. × 600.

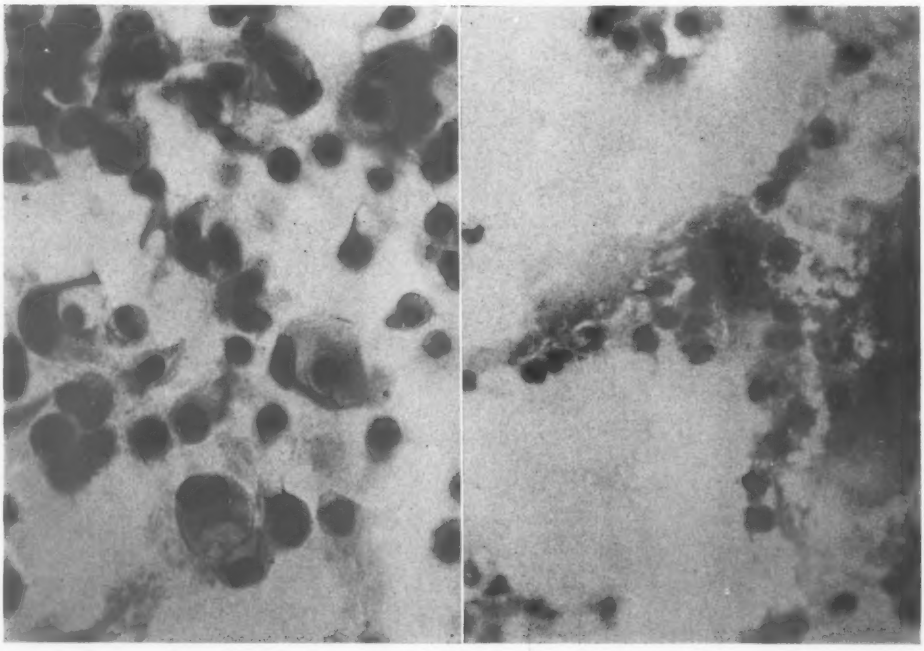
FIGS. 10 and 11. Examples of multinucleation in this type of carcinoma. × 600.

FIG. 12. Cluster strongly indicating anaplastic or oat-celled type. × 600.

FIG. 13. Vacuolated cells very typical of bronchogenic adenocarcinoma. × 600.

FIG. 14. Cells probably indicating pleomorphic bronchogenic carcinoma. × 600.

FIG. 15. A cluster suggesting terminal bronchiolar origin. × 600.



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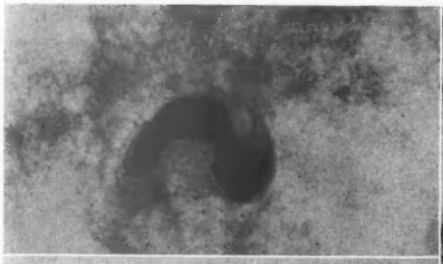

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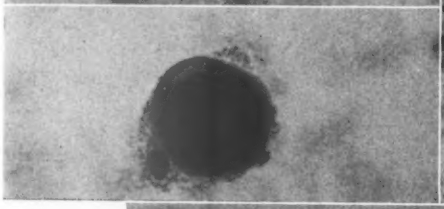

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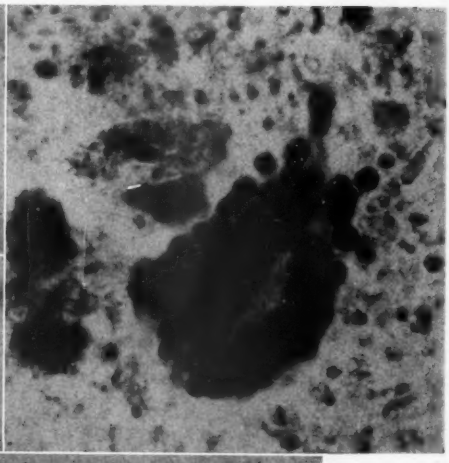

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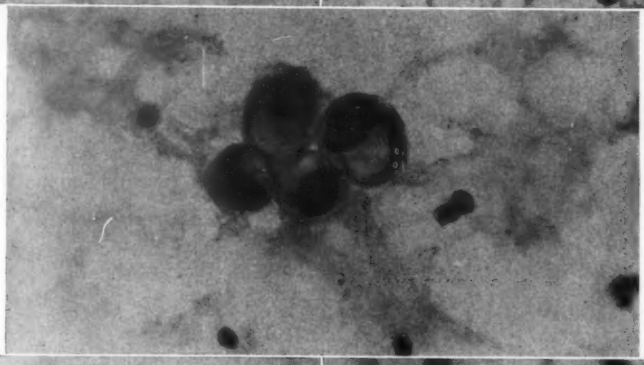

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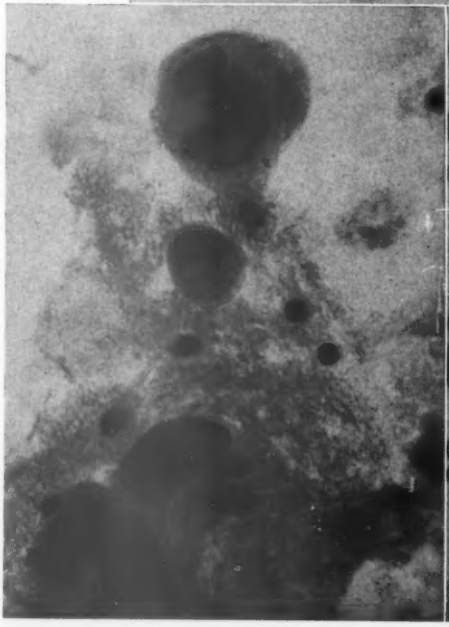

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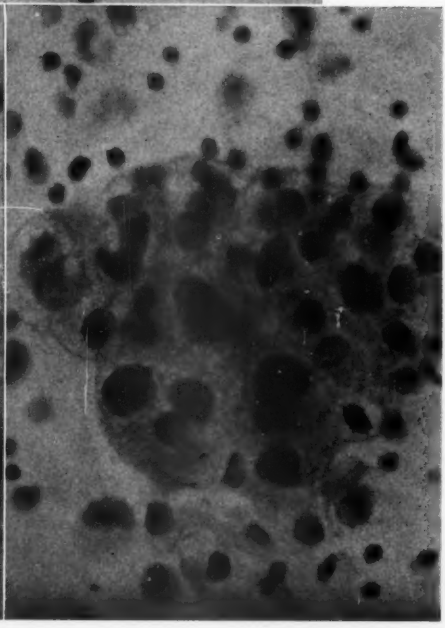


Figs. 16 to 20 are examples of cells from ovarian carcinoma. Figs. 21 to 24 are examples of cells from gastric carcinoma.

FIG. 16. Papilliform proliferation sphere. × 600.

FIG. 17. Cluster illustrating sham double contour of cells. × 600.

FIG. 18. Large binucleate signet-ring cell, unfortunately rarely found in smears of serous fluids. × 600.

FIG. 19. Finely vacuolated cells in copious exfoliation. × 600.

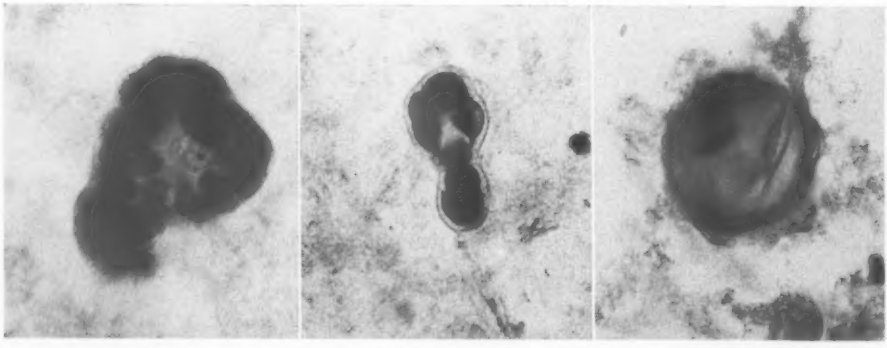
FIG. 20. Nondescript cells too often found in smears of ovarian carcinoma. They account for many mistakes in diagnosing source. × 600.

FIG. 21. Cluster of small typical cells from gastric carcinoma. The nuclei are almost pyknotic. × 600.

FIG. 22. Enormous vacuolated cell. × 600.

FIG. 23. Fusiform epithelial cells noted only in gastric carcinomas in this series. × 600.

FIG. 24. Sausage-shaped cells. × 600.



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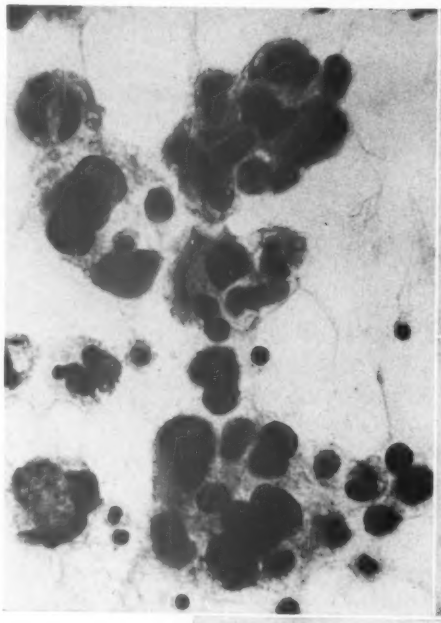

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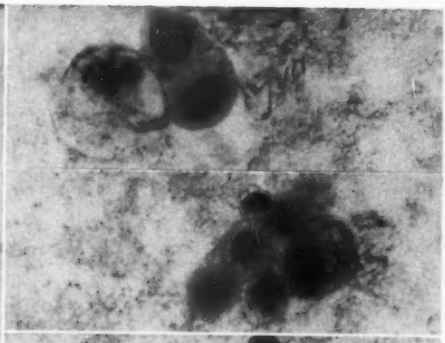

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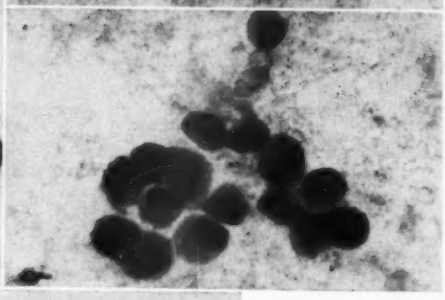

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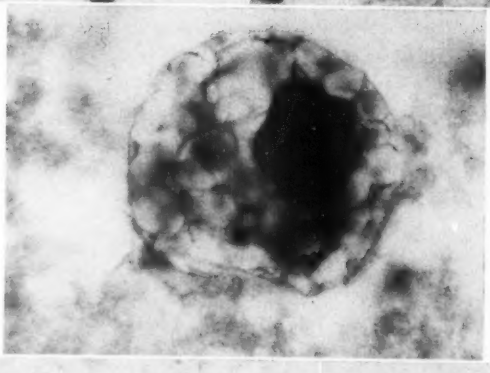

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Fig. 25 is from a smear of gastric carcinoma.

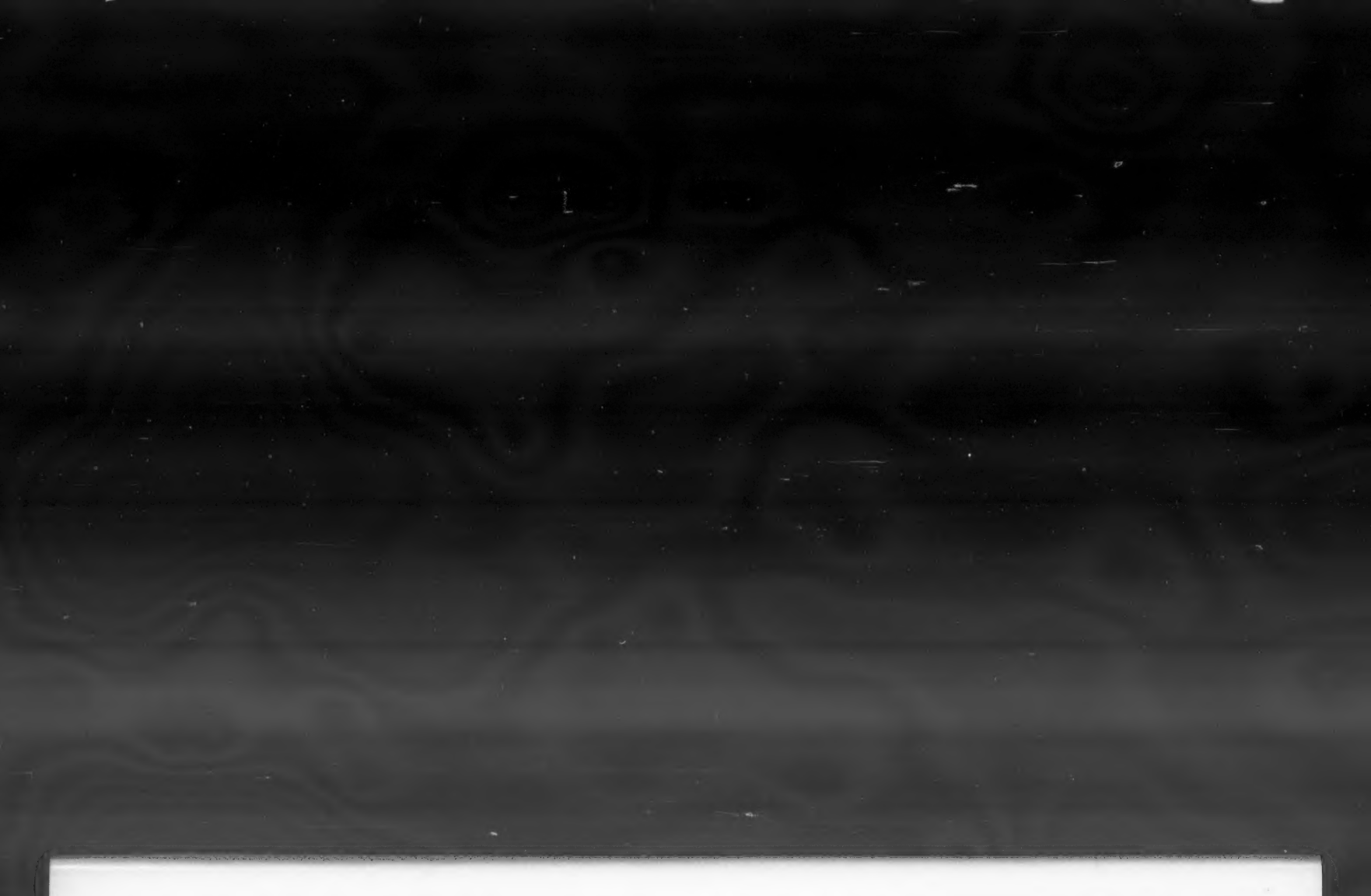
FIG. 25. Proliferation sphere that is a rarity in gastric carcinoma and would probably cause a mistaken diagnosis of mammary or ovarian carcinoma. × 600.

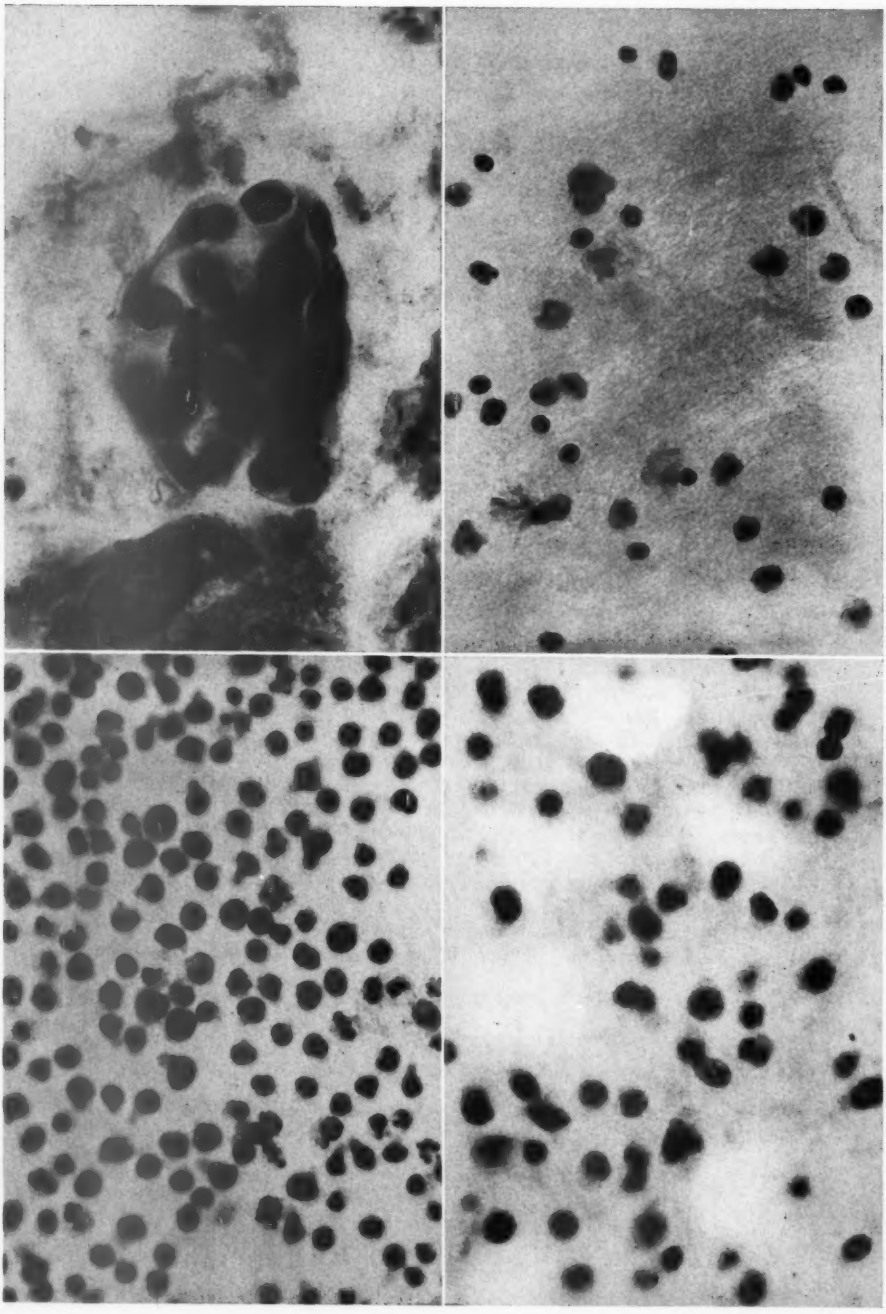
Figs. 26 to 28 show cells from cases of lymphoma.

FIG. 26. Typical appearance of cells of Hodgkin's disease. Of note are the pallor of the cells and their size as compared with lymphocytes. × 600.

FIG. 27. Typically copious exfoliation in lymphosarcoma. × 600.

FIG. 28. Larger lymphoid cells from lymphosarcoma. × 600.





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A PRESUMABLY BENIGN TUMOR AND A PROVED MALIGNANT TUMOR OF THE CAROTID BODY *

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One of the two tumors of the carotid body which are reported in this paper is interesting with respect to its histogenesis, the other, because of its unusual course.

According to Goormaghtigh and Pannier,[1] the carotid and aortic bodies (Penitchka) are true glomi, the arteriovenous channels of which are in close contact with sensitive, non-chromaffin paraganglionic cells. The latter are enclosed in a cellular capsule and connected with afferent neurofibrils. The existence of arteriovenous anastomoses in the carotid body is denied by LeCompte[2] and Hollinshead.[3] On the other hand, according to Van Campenhout,[4] the paraganglionic nature of the distinctive cells of the carotid body is not proved.

The histologic structure of the tumor in case 1 emphasizes the peculiar features of its vascularization and the paraganglionic characteristics of some of its cells.

The clinical history and the post-mortem findings of the second case illustrate the fact that, exceptionally, some neoplasms of the carotid body are malignant.

Report of Cases

Case 1

A male patient, 52 years old, had complained for about 1 year of a swelling in the upper part of the neck on the left side. Deglutition was normal, but he complained of irritation of the throat on the left side and of neuralgic pain radiating toward the left ear. Surgical removal was advised. At operation in June, 1952, a firm, pinkish, ovoid, encapsulated tumor the size of a pigeon's egg was found behind the bifurcation of the common carotid artery. In front of the artery lay an enlarged soft lymph node of the size of a hazelnut. Owing to the firm connection with the carotid artery, the latter had to be resected (Drs. Morelle and Simonart). The lymph node was removed at the same time. Postoperative recovery was uneventful. At present, the patient still complains of fatigue after intellectual work, of a sensation of cold in the left temporal region, and of a dull pain above the left clavicle. The vision of the left eye is corrected by ¼ of dioptry. Ocular tension is the same in both eyes.

We had the opportunity of studying serial sections of the whole tumor fixed in Bouin's solution through the courtesy of Professor E. Van Campenhout (Louvain).

Histologic Findings

A fibrous capsule of variable thickness invested the tumor and was connected by fibrous bands with a collagenous core almost devoid of

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neoplastic cells. The bulk of the latter were disposed around this core. These cells formed highly vascularized lobules which, according to their size, histologic features, and vascularization, were classified in three categories:

(a) Small lobules irrigated by the branches of a single arteriole and surrounded by a network of small vessels, which, for the greater part, were not ordinary capillaries because their endothelium was supported by smooth muscle cells. In these small lobules the tumor cells and their nuclei usually were spherical (Fig. 1). Each cell or group of several cells was encased in a network of fine collagen fibers resembling the pericellular capsule observed by Meijling[5] and Goormaghtigh and Pannier[1] in the normal carotid or aortic bodies. Between the spherical cells and their collagen capsules were inserted capsular cells with oblong nuclei (Figs. 2 and 3). Such cellular groups constituted functional units resembling the normal pattern (compare Figs. 2 and 3 with Figs. 1 and 2 of the paper of Goormaghtigh and Pannier).

(b) Larger lobules composed of several units of type (a). In the lobules of types (a) and (b) anaplasia was slight. Only a few cells had lost their spherical shape and their capsule. No special staining techniques were applied for demonstrating neurofibrils.

(c) Still larger lobules subdivided into cell groups of an alveolar pattern (Fig. 5). The closely packed cells varied in size and shape. Their cytoplasm was abundant, acidophilic, and finely granular. Their nuclei were large, moderately chromatic, and their irregular shape contrasted with the perfectly spherical shape usually observed in the less anaplastic lobules. The pericellular capsule was absent. The cell boundaries became indistinct. On the other hand, the pattern of vascularization was the same as in lobules (a) and (b). The smallest blood vessels were not true capillaries. Their walls were built up by either a continuous or discontinuous layer of smooth muscle cells which supported the endothelium. In some lobules of the (c) category the vascular wall which was in contact with the neoplastic cells consisted only of endothelium while on the opposite side it contained smooth muscle cells (Fig. 5). Venules ran close to the cell groups a, b, and c. They were connected with the smaller vessels irrigating the neoplastic lobules. In the fibrotic core of the tumor, the latter underwent autolysis while the surrounding blood vessels persisted much longer. As a result, some areas resembled a hemangioma, and a close examination showed that the nuclei of the vascular cells varied considerably in size (Fig. 4).

The fibrotic core of the tumor contained large ovoid lamellar structures, the axis of which was occasionally occupied by a nerve bundle

(Fig. 6). More often, an aggregate of coarse collagen fibrils was all that remained of a nerve structure. In other words, the nerve bundles of the carotid body, from which the tumor was derived, degenerated while the paraganglionic and vascular cells proliferated jointly and became gradually anaplastic. In their turn the neoplastic cells underwent regression in the fibrotic core of the growth. The lymph node adjacent to the tumor did not contain neoplastic cells.

Case 2

A male patient, 52 years old, consulted a specialist in 1943 because deglutition had become difficult 3 weeks previously. A hard tumor of the size of an egg could be palpated in the upper right region of the neck. The right half of the tongue and the right vocal cord were paralyzed (syndrome of Tapia). A small fragment for biopsy was sent to our department and registered under no. 23688. At the time, no detailed clinical data were forwarded to the pathologist. The biopsy piece contained a cluster of atypical polygonal cells adjoining lymphoid tissue. No firm diagnosis was made; the possibility of a metastasis of an epithelioma of unknown origin was not ruled out. Deep radiotherapy was applied. The swelling receded markedly. The patient gave up treatment voluntarily. In 1944 he was readmitted to the radiotherapeutic department of the Tumor Center. By that time the growth had increased in size, was bulging into the pharynx, and pushing the soft palate upward. From 1944 to 1949 it was irradiated at regular intervals. Fistulization occurred (skin). In 1949 the patient began to complain of backache. An osteoplastic metastasis in the second lumbar vertebra was discovered and irradiated. Early in 1950 the main tumor extended to the right supraclavicular region, involving the plexus brachialis. In January, 1951, roentgenologic examination revealed pulmonary metastases and involvement of the fourth lumbar vertebra. Telecurietherapy in 1952 was of no avail. The patient died in August, 1952.

Post-Mortem Examination

At necropsy (no. 7656) 40 hours after death, the body weighed 35 kg. The organs of the neck and the main tumor were removed *in toto*. Their dissection revealed a hard ovoid tumor, 7 by 10 by 20 cm., of which the medial side, in its lower part, presented a groove containing the right common carotid artery. The bifurcation of this artery was situated at about 1 cm. above its penetration in the tumor mass. The external carotid artery was patent; the internal carotid artery showed a kink inside the tumor and had undergone thrombosis (Fig. 7). The tumor bulged into the pharynx, the wall of which was easily separated from the neoplastic mass. There was neoplastic involvement of the cervical, mediastinal, and para-aortic lymph nodes, the latter being very much enlarged at the level of the second lumbar vertebra. A grayish metastatic nodule was surrounded by red bone marrow in the body of this vertebra and in that of the fourth lumbar. Numerous spherical metastatic nodules (1 to 1.5 cm. in diameter) were found in the lungs. Some were grayish, some pinkish. There was purulent

tracheobronchitis and passive congestion of the lungs. The heart weighed 300 gm. in spite of severe emaciation; the heart cavities were dilated. The changes of nutmeg liver were present to a slight degree.

Anatomical Diagnosis. Carotid body tumor metastasizing to the cervical and mediastinal lymph nodes, the lungs, and the second and fourth lumbar vertebrae. Subacute passive congestion of the liver and lungs. Purulent tracheobronchitis. Cachexia.

Histologic Findings

The histologic structure of the primary tumor as well as of its metastases was that of an alveolar carcinoma composed of epithelial cell groups of variable size, enclosed in an uninterrupted meshwork of capillaries supported by collagen (Fig. 8). The cells varied in size and shape. In some of the metastatic nodules they often were elongated. There was no trace of pericellular capsules. The collagen framework was coarse in the primary tumor, perhaps as the result of prolonged irradiation. No perineural lamellar structures comparable to those described in the tumor of case 1 existed in the primary tumor. Instead, one sometimes found round, hyaline, collagen nodules which corresponded perhaps to a more advanced stage of regression of the nerve bundles. In some of the pulmonary metastases the capillary network was in close contact with the neoplastic cells with hardly any interposition of collagen. Macroscopically, these nodules appeared pink. Those in which fibrosis was more marked and in which a central collagenous core existed were white or gray. In the metastatic nodules of the vertebrae the pattern of vascularization resembled that of the tumor of case 1, as can be clearly shown in reticulin preparations (Fig. 8).

Comment

The fact that the smallest vessels of the tumor of case 1, which mimics in some of its parts the structural pattern of the normal organ, are not capillaries, supports to a certain extent the opinion of Goormaghtigh and Pannier who contended that, with regard to its mode of vascularization, the carotid body is a glomus in the true sense of the word.[1,6,7] In the glomi of the skin the active arteriovenous anastomoses are composed of an arterial segment and a venous segment. The walls of the latter are thin and supported by smooth muscle cells. The cells of the normal carotid body cells are connected with the venous segment. This is also the case in some parts of the tumor of case 1, as illustrated in Figure 5.

In the more anaplastic tumor of case 2 the pattern of vascularization is simpler, except in the metastatic nodule of the lumbar vertebrae

where some of the distinctive features of the tumor of case 1 reappear. The tumor of case 2 contains no arterioles but only capillaries which form a perfect network. Consequently there is no necrosis. Few carcinomas are so adequately vascularized. The absence of necrosis is, in our view, of some diagnostic value.

We submit that the intimate relation of the normal carotid body cells with the venous section of the arteriovenous anastomoses on the one hand and with afferent neurofibrils on the other (DeCastro,[8] Goormaghtigh and Pannier[1]) is compatible with the chemoreceptor function established by Heymans and Bouckaert.[9] In this respect the morphologic changes observed by Langer[10] are suggestive.

The nature of the distinctive cells of the carotid body has not been established definitely (*cf.* Van Campenhout[4]), but there is little doubt that they are derived from the neural crest at the same time as the ganglionic cells of the autonomous nervous system and the intra-adrenal and extra-adrenal paraganglionic cells. The bulk of the carotid body cells are non-chromaffinic (Mönckeberg,[11] Goormaghtigh and Pannier[1]), but chromaffinity should not be considered as the distinctive feature of all paraganglionic cells. One of us[12] has found non-chromaffinic paraganglia associated with the vagus nerve. Incidentally, it should be borne in mind that in a normal carotid body a few true sympathetic chromaffin cells are present (Mönckeberg; Goormaghtigh and Pannier). Mönckeberg has even traced some in carotid body tumors. Nevertheless, the bulk of the cells of the aortic and carotid body differ from the paraganglionic cells referred to, chiefly because they, or small groups of them, are enclosed in a cellular capsule (Meijling,[5] Goormaghtigh and Pannier). This brings them closer to the true ganglionic cells. In other words, they occupy an intermediary position between the ganglionic cells of the autonomous nervous system and the chromaffin or non-chromaffin paraganglionic cells associated respectively with the sympathetic and vagus nerves.

Should they be called ganglionic cells of a special type (Meijling[5]) or sensitive paraganglionic cells (Goormaghtigh and Pannier[1])? The question dwindles to one of terminology.

Whatever the outcome of this discussion, it is an interesting fact that in the tumor of case 1 some of the tumor cells retained their cellular capsule. We believe that this has not been observed before.

As a rule, according to Mönckeberg,[11] Bloom,[13] and LeCompte,[2] the neoplastic cells are packed in well delineated masses. The tumor of case 2 corresponds to this adenoma-like type. LeCompte, moreover, distinguished an "angioma-like" type. Some of the anaplastic areas of the tumor of case 1 acquire such features as the result of the re-

gression of the atypical paraganglionic cells and the persisting proliferation of the vessels.

We consider that the characteristic feature of a tumor of the carotid body is the joint neoplastic proliferation of its vascular and paraganglionic components. The smaller vessels may retain a differentiated media; if not, they continue to form an adequate meshwork enclosing the neoplastic islets. When the vascular proliferation has the upper hand, a tumor of the type of a hemangioma is the result.

Of interest in case 1 is the mode of regression of the nerve bundles, which leads to the formation of lamellar connective tissue knobs. As to the terminal neurofibrils, we have made no attempt to stain them with specific techniques; they probably disappear in the more anaplastic tumors. Chase[14] found nerve fibers to be plentiful in the tumor of the carotid body which he reported. Bertrand and Sauvage[15] detected, with the aid of the Bielschowsky and Gros techniques, nonmyelinic neurofibrils supported by Schwann cells in the interlobular connective tissue septa and a more delicate neurofibrillar network inside the neoplastic lobules. However, the pattern of innervation is not as perfect as that of the normal organ as first outlined by DeCastro.[8]

From a clinical point of view our case 2 is exceptional. Its course was malignant but extended over a period of 9 years. In many of the recorded cases of malignant carotid body neoplasms there is room for doubt about the true nature of the primary tumor (Willis[16]). Only after a complete necropsy can a metastatic deposit in the carotid lymph nodes from a primary tumor elsewhere be ruled out. No reported case of a malignant tumor affords such a proof, according to Willis[16] and LeCompte.[2]

On the other hand, Harrington, Clagett, and Dockerty[17] have put the incidence of malignant neoplasia as high as 50 per cent. Their conclusions were based exclusively on the histologic examination of twenty tumors observed in the Mayo Clinic. They used as criteria of malignancy the presence of giant cells, mitotic figures, hyperchromatic nuclei, multiple nucleoli, and invasion of the capsule. The follow-up of these patients, however, shows that these criteria cannot be relied upon. With regard to the histologically malignant series, only in three instances was the postoperative course such as to arouse a clinical suspicion of malignancy. One concerned a female patient, 35 years old, who, some time after the operation, developed epilepsy and died. A brain metastasis was suspected but not proved since a post-mortem examination could not be performed. Two patients died respectively 8 and 10 years after operations. Symptoms developed which were con-

sidered as evidence of a recurrence, but again post-mortem control was impossible. The other patients were still alive when the report was published. One had already survived 15 years; another, 11 years. Does the history of these patients point to the efficiency of surgery or the failure of the histologic criteria? Of the 10 patients with histologically benign tumors, 5 were followed up: all were in good health after periods varying from 4 to 14 years. In fact, the survival rate seems to have been nearly the same in the histologically malignant series and the histologically benign series.

From the perusal of the 257 reports of tumors of the carotid body (including the recent cases of Brown and Fryer[18] and Pemberton and Livermore[19]) which we were able to collect, it seems probable that only four carotid body tumors have given rise to distant metastases; namely, those recorded by Bérard and Dunet[20] in 1923, Duroux and Rollin[21] in 1927, Donald and Crile[22] in 1948, and our own case 2. LeCompte[2] cast some doubt on the report of Donald and Crile because no post-mortem evidence was available. The same can be said of the case of Duroux and Rollin which is the least convincing of the four. However, the existence of metastases reported by Donald and Crile was based on roentgenologic examination; the metastases were situated in the bones, a similarity with our case 2 which was verified at necropsy.

In early papers on the subject mention was made of the involvement of the cervical lymph nodes (Kretschmar,[23] 1893, and Kopfstein,[24] 1895), but histologic data are lacking. Nothing more has been published since about the subject. In our case 1 the enlarged cervical node close to the tumor was not invaded by tumor cells. If the four cases of distant metastases referred to are taken into account, the malignancy of carotid body tumors should not exceed 1.5 per cent (calculated on 257 cases).

The post-mortem findings of our own case show that the extension of the neoplastic process takes place along the lymphatic route in a craniocaudal direction, involving a majority of the mediastinal lymph nodes, the lungs, and two vertebrae. The malignant clinical course is slow in a majority of cases (Table I). From a survey of the literature also emerges the fact that the great majority (252 of 257) of tumors have been operated upon. Would some of these not have given evidence of a low grade of malignancy if they had been left alone, as in our case 2? Perhaps, after all, the contradiction between the histologic criteria of malignancy and the benign clinical course is not real.

Since malignant behavior remains a possibility, carotid body tumors

should be removed as a security measure. But if their removal requires the resection of the carotid arteries, the risk of cerebral damage will have to be put in the balance against the apparently small risk of malignancy. In such an event, the size of the tumor and its local inter-

TABLE I

Reported Cases of Carotid Body Tumors with Distant Metastases

	Survival	Mediastinal lymph nodes	Vertebra	Lungs	Ribs
Bérard-Dunet,[20] 1923	3 years		−	+	−
Duroux-Rollin,[21] 1927	1 year	+	−	+	−
Donald-Crile,[22] 1948	11 years	−	+	−	+
Goormaghtigh-Pattyn, 1954	9 years	+	+	+	−

ference with the function of vital organs will determine the therapeutic decision.

The history of our second case proves that radiotherapy of a malignant carotid body tumor is not to be recommended.

SUMMARY

Two cases of unilateral tumor of the carotid body are reported. Both patients were male. In both cases the tumor became manifest during the 52nd year.

The histologic structure of some parts of the first tumor mimicked that of the normal organ: pericellular capsules; features of vascularization which resembled those of active arteriovenous anastomoses. A peculiar type of regression of the nerve bundles was noted. The second tumor was anaplastic and resembled an alveolar carcinoma. Adequate vascularization and absence of extensive necrosis are distinctive features of anaplastic carotid body tumors.

One of our cases, which was not operated upon, had a malignant course extending over 9 years. Metastases were found in cervical, mediastinal, and retroperitoneal lymph nodes, the lungs, and lumbar vertebrae.

After a review of the literature, the incidence of malignancy of carotid body tumors, most of them operated upon, is estimated at 1.5 per cent.

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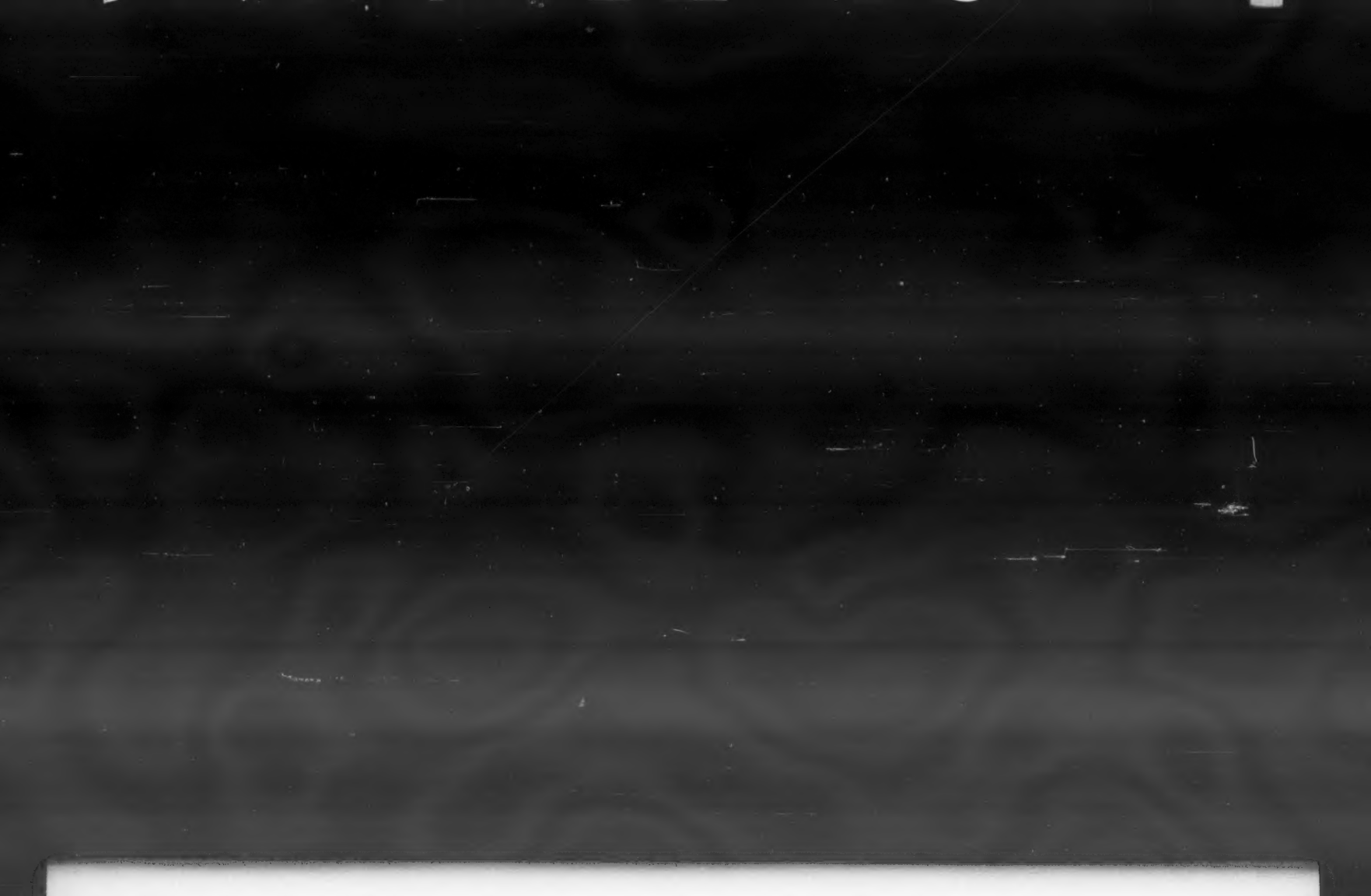
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Carotid body tumor of case 1. General view of the least anaplastic area. Clusters of paraganglionic cells often are surrounded by a capsule. In the center a venule is seen sectioned longitudinally. At the lower end of this venule there is an isolated, round, paraganglionic cell with a capsular cell (black dot near a clear round nucleus). Bouin's solution, Masson's trichrome stain. × 150.

FIG. 2. Near the extremity of a venule seen in longitudinal section there is an isolated paraganglionic cell surrounded by a capsule. The oblong, dark nucleus of a capsular cell seems to touch the clear round nucleus of the paraganglionic cell. On the left of this cell is a small arteriole with large oblong nuclei. Bouin's solution, Masson's trichrome stain. × 1125.



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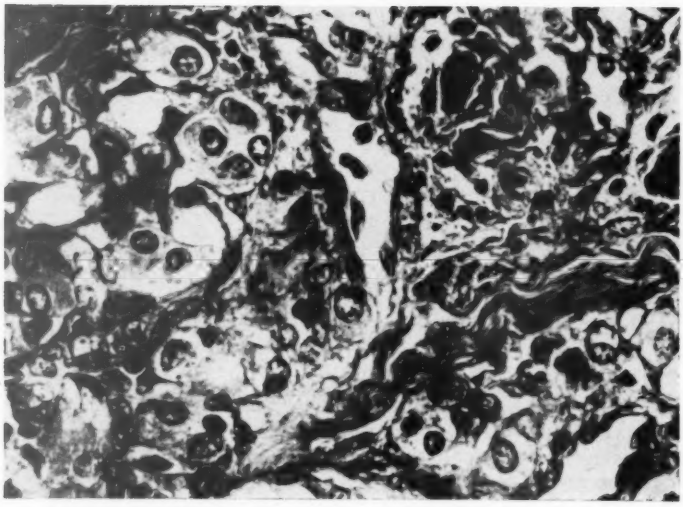

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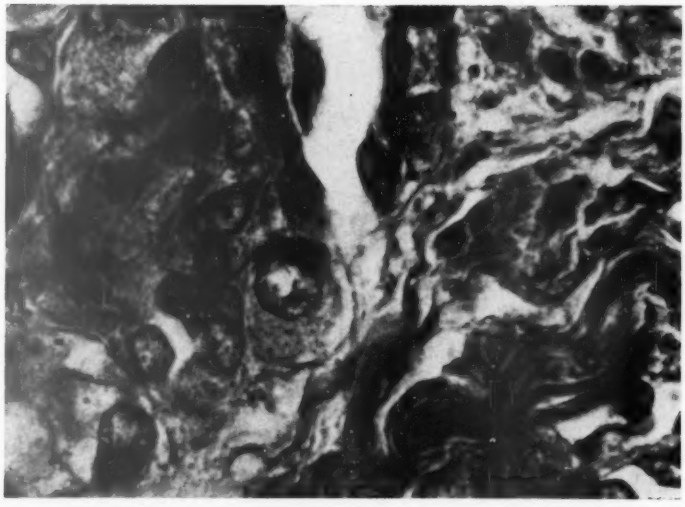

FIG. 3. Drawing on a larger scale of paraganglionic cells in the same area of the tumor of case 1. Of note are the capsule and capsular cells. The endothelium of nearly all the small vessels is supported by smooth muscle cells. Mast cells are present also. Bouin's solution, Masson's trichrome stain.

FIG. 4. Degenerating neoplastic cell groups in the collagenous core of the tumor of case 1. Intact arterioles. Hemangioma type. Bouin's solution, Masson's trichrome stain.

FIG. 5. Anaplastic area of the tumor of case 1. There is a pseudo-capillary vessel in the upper right-hand corner. An endothelial cell is in contact with the neoplastic cell group. On the opposite side of the vessel the endothelium is supported by a group of smooth muscle cells. Bouin's solution, Masson's trichrome stain.

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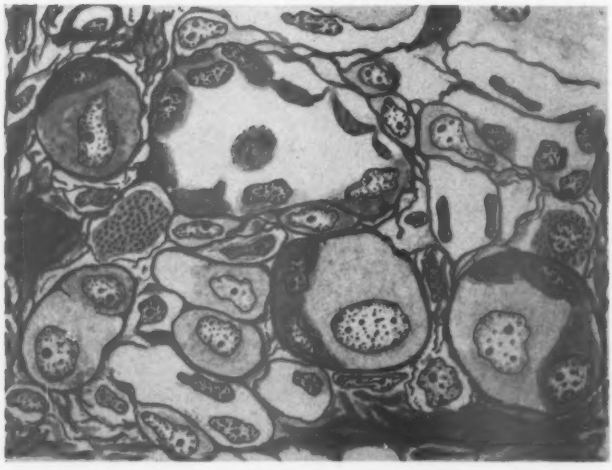

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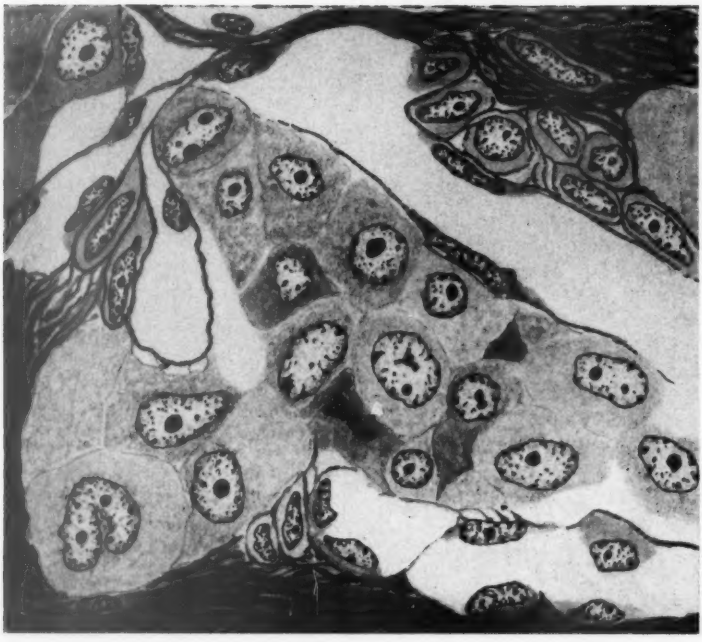
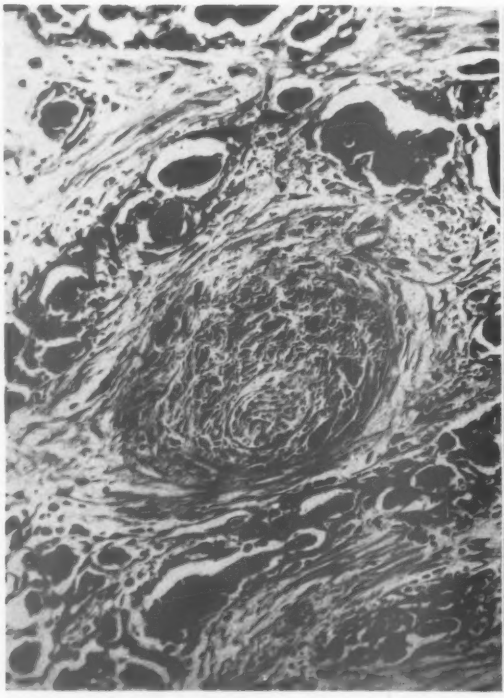


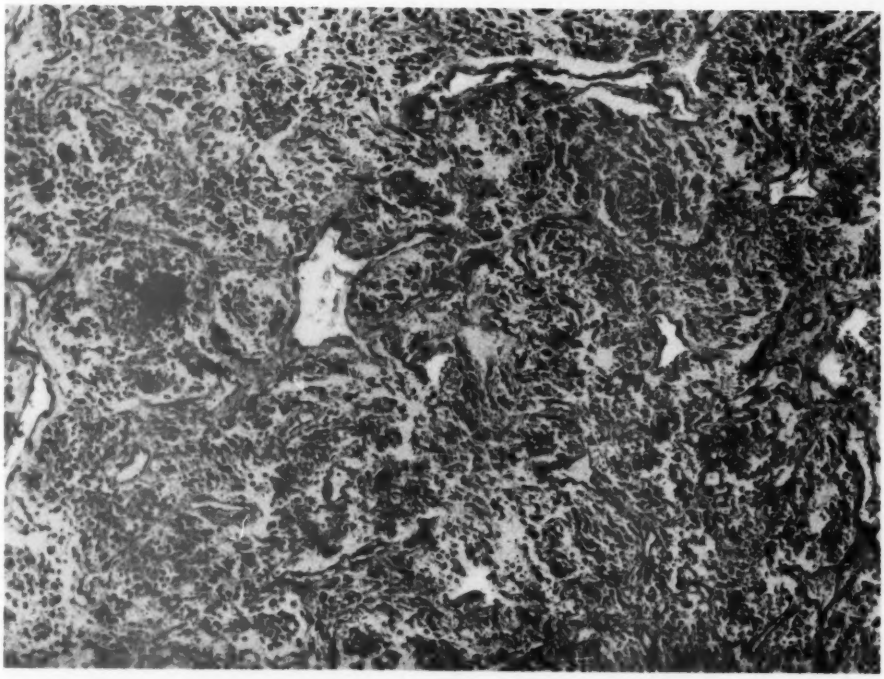
FIG. 6. Fibrotic core of the tumor of case 1. In the center is a round lamellar structure in which a nerve bundle can be recognized. Around this collagenous knob, dark-stained anaplastic cords can be seen. Bouin's solution, Masson's trichrome stain. × 150.

FIG. 7. Carotid body tumor of case 2.

FIG. 8. General view of the carotid body tumor of case 2. Formalin fixation, hematoxylin and eosin stain. × 50.

FIG. 9. Metastasis of the tumor of case 2 in the fourth lumbar vertebra. The neoplastic cords are greatly altered by post-mortem autolysis and decalcification but the development of the vascularization is clearly shown by the reticulin stain. Formol fixation, Foot's silver stain. × 100.





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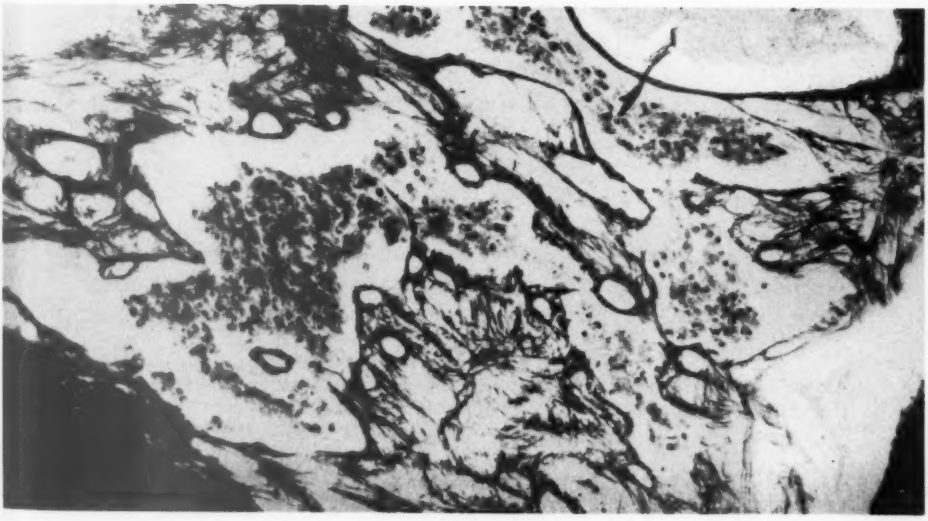

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BLISTER FORMATION AND TISSUE TEMPERATURE IN RADIANT ENERGY AND CONTACT BURNS *

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Recent developments in military weapons have so increased the possibility of exposure to high intensity radiant thermal energy that the problem of flash burns is attracting considerable attention. In a study of burns of pigs' skin from exploding magnesium, Pearse, Payne, and Hogg[1] obtained evidence that the flash burn differs from the ordinary moderate temperature burn. Evans,[2] on the other hand, found the clinical course of patients burned by exploding magnesium to be similar to that of patients with ordinary burns. Experimental evidence from radiant burns of rat skin, reported from this laboratory, indicates that in many respects contact and radiant thermal burns are similar, the chief difference being a possible increase in edema at the margins and base of the more slowly produced contact burn.[3,4] It seems likely that if differences between radiant and contact burns exist, they are functions of the tissue temperatures attained and of the duration of temperature elevation, and are not related to any unique properties associated with the manner of delivery of energy. This concept requires modification if the radiant energy concerned has a large component at wave lengths which initiate specific photochemical reactions.

In this investigation burns were produced by high intensity radiant thermal energy and by contact with hot water. The median effective radiant energy, EC_{50}, and the median effective water temperature, ET_{50}, for the production of blistering on either or both sides of the ear of a rat were determined for various durations of exposure. Following evaluation of EC_{50} and ET_{50}, subcutaneous temperatures were measured at or near these values for exposure times of 1.0, 4.0, and 8.0 seconds.

METHODS

A modified Navy 36 inch carbon arc searchlight was used as the thermal radiation source.[3] More than 95 per cent of the energy from this source was between 3500 Å and 25000 Å. Exposures were made through an aperture 1 cm. in diameter, placed at the focus of a paraboloid mirror which intercepted the nearly parallel rays of the search-

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light beam. In order to secure a constant aperture starting temperature for each exposure, water at 30° C. was circulated through the hollow copper aperture plate. The exposure times were controlled by sweeping the searchlight beam past the mirror at various rates. For the conditions of exposure used, the energy pulse shape was symmetric and was essentially triangular. Exposure time was defined as the time which would be required to deliver the total energy dose, assuming the delivery rate to be maintained at the maximum attained during the exposure interval. Continuous monitoring of the beam was accomplished by means of a calorimeter* at the focus of a second paraboloid mirror which intercepted a portion split from the main beam by a sheet of plate glass. The monitor values were related to the energy delivered at the exposure focus by means of a second calorimeter at the exposure focus. The beam intensity was varied by the use of wire and/or sheet metal screens of various transmission values.

For some exposures a shutter mechanism was employed to control the exposure time.[5] The shutter consisted of two pneumatically operated polished aluminum blades near the exposure focus. The movement of one blade exposed the area to be burned; movement of the second covered the exposed area. The travel time of each blade was approximately 8 milliseconds. The shutter was electrically linked to the iris diaphragm on the searchlight which remained stationary, so that the blades were only briefly subjected to direct radiation; thus minimizing blade temperature rise and re-radiation to the surface to be exposed. This method provided an approximately square energy pulse. However, since there was no continuous energy monitoring available for use with the shutter mechanism, most observations were made with the sweep technique.

For hot water burns the animals were placed behind an aperture 1 cm. in diameter in a lucite panel ⅛ inch thick, and water from a thermostatically controlled bath was pumped from the end of a rubber tube having an inside diameter of ⅜ inch at a pressure resulting in a flow of 2.7 l. per minute against the surface exposed in the aperture. A solenoid arm in front of the panel and attached to the tubing served to direct the flow toward or away from the aperture as desired. The end of the tube moved in a plane 1 cm. from and parallel to the aperture. The water flow was prevented from impinging on the panel, except during the exposure phase, by a stationary sheet metal guard, which was so placed that water did not impinge on the panel or the

* All energy values are given in gram calories per square centimeter, as measured by a black body calorimeter and refer only to incident radiation. No correction has been made for reflection or scattering.

surface to be exposed until the tubing pointed almost directly toward the aperture. In this manner the temperature in the center of the aperture, as indicated by a fine wire thermocouple, was brought from the starting air temperature to the temperature of the heated water within 0.02 seconds after the first perceptible temperature rise. The lucite panel was maintained at the proper temperature by circulating water through a jacket around the aperture. The temperature of the panel near the aperture was indicated continuously by means of an attached thermocouple.

Subcutaneous tissue temperatures were measured with bare wire thermocouples made of 60/40 gold-palladium, 0.0013 inches in diameter, and copper, 0.0006 inches in diameter, as shown in Figure 1. To construct a thermocouple, 1.5 cm. lengths of the wires were held by copper clamps under a dissecting microscope. The short ends protruding from the clamps were clipped to a length of about 1 mm., cleaned with 10 per cent HCl, then with acetone, and, finally, were electrically butt welded by condenser discharge. The welded couple was re-washed with acetone and coated with light oil. Placement of the thermocouple was effected by inserting a 1 cm., 27 gauge hypodermic needle beneath the skin. The bare couple was threaded through the needle and the latter withdrawn leaving the couple in place. Temperature measurements were made between 1 and 2 hours after introduction of the couple. Microscopic sections of some of the ears containing inserted thermocouples showed the couples to be in relatively uniform position about 0.5 mm. beneath the exposed medial surface between the ear cartilage and the epidermal layer of the lateral surface, as shown in Figure 2. In all burns the medial surface of the ear was directly exposed at the burn aperture. Most of these ears had a thickness near the base of about 0.7 to 1.0 mm. Gross and microscopic examination of ears in which thermocouples had been inserted, but no other manipulation made, showed that the injury caused by the insertion was usually indicated by only a slight edema and the release of a few erythrocytes in the vicinity of the couple.

An oscillographic recorder and a strip chart recording potentiometer were employed to measure output of the thermocouple circuit. Measurements of maximum temperature were obtained from both records. The oscillographic record allowed a better time resolution and was used for estimation of the time to reach maximum temperature and for the time-temperature curves.

In the case of hot water burns, the time of starting exposure was defined as the time of arrival of hot water at the aperture and was indicated by a themocouple in the aperture. For the sweep technique

radiant burns, zero time was defined as the time of the first detectable response of the subcutaneous thermocouple. That the latter definition is adequate is indicated by the fact that for the shutter technique, with radiant exposures of 3 to 4 calories per square cm. per second, the first perceptible response of the thermocouple occurs within about 0.01 seconds after activation of the shutter circuit.

The following exposure technique was used. Two-month-old Sprague-Dawley albino rats were anesthetized with sodium pentobarbital 1 to 2 hours prior to burning. A gummed tape, in which a hole the size of the exposure aperture had been punched, was attached to the lateral surface of each ear. When a temperature measurement was to be made, the thermocouple was next inserted. The ear was then placed in position at the aperture and held essentially flat by means of the tape. In most cases both ears of a rat were exposed consecutively. Three to 4 hours after exposure each ear was examined on both sides and the presence or absence of a blister containing demonstrable fluid was determined.

A total of 56 to 150 animals, 112 to 300 burns, were observed for blister formation at each exposure time for each type of burn. Additional animals were used for subcutaneous temperature measurements and for "water-backed" burns, those in which water was caused to flow on the unexposed side of the ear. Throughout this paper all references to radiant burns refer to air-backed burns unless otherwise specified. Determination of each EC_{50} and ET_{50} value is based on observations made on 3 or more different days. For estimation of each mean maximum temperature, observations were made on 2 to 4 different days. The values and 95 per cent confidence intervals for the median effective water temperature (ET_{50}) and the median effective radiant energy (EC_{50}) for production of blistering on the exposed and non-exposed sides of the ear were calculated by the method of Litchfield and Wilcoxon.[6] The mean and standard deviations of the initial and maximum temperature values for each set of conditions studied, exposures of 1.0, 4.0, and 8.0 seconds for the arc and for the hot water, were calculated. For each exposure time the tissue temperature data were plotted as a function of time after onset of exposure, using the mean of the values obtained from two to four separate measurements.

In determining subcutaneous temperatures at the EC_{50} for radiant thermal energy blistering, it was found that the values varied from day to day, apparently as a function of the initial skin temperature. When the temperature of the burning room was low, the initial and maximum ear temperatures tended to be low. Furthermore, since the inherent variations of the output of the carbon arc source prevent

accurate control of the energy, the energy actually delivered to the ear often varied slightly from the desired amount. Consequently, the tissue temperatures found for the radiant burns were corrected to a common initial temperature and were also corrected for the difference between energy delivered and the EC_{50} value for the particular exposure time.* In each case the amount of energy applied was approximately equal to the EC_{50} value. All temperature values employed in computing mean maximum temperatures for radiant burns have been so corrected. The curves for temperature *vs.* time after onset of exposure are shown only to indicate the general nature of the time-temperature response, and the data used were not corrected. In the case of the hot water burns, there was no basis for correcting for initial temperature.

Results

Pathology of the Burn Lesion

It was found for the range of exposures studied that, when the exposure time is maintained constant and the amount of radiant energy applied to the ear increased stepwise, the following levels of gross pathologic change occur. The first perceptible injury is a transient erythema. At a slightly greater energy the epidermal-dermal adhesion becomes impaired and, if friction is applied, the epidermis slips from the dermis. The next distinguishable pathologic entity is characterized by true vesication; the vesicles are well formed within 1 to 3 hours after the injury and contain a clear colorless fluid. At energies somewhat above the vesication level, full skin thickness necrosis of the burned area results. In the more severe burns there is a peripheral gradation of tissue damage in which changes characteristic of lesions produced at lower energies are seen.

To appraise the vascular status of the lesion, trypan blue was given intravenously shortly after burning. When trypan blue is injected, the normal skin becomes moderately blue. If energy sufficient to produce vesication has been applied, the burned area becomes an intense blue. In full thickness necrosis of the skin, the necrotic area remains white.

Figures 3 and 4 show two ears of rats approximately 3 hours after exposure to heated water; blistering resulted on both sides of each ear. One burn, Figure 3, was the result of an 8.0 seconds contact with 56.0° C. water, while the other, Figure 4, was caused by exposure to 59.0° C. water for 4.0 seconds. Elevation of epidermis, the presence of a subepidermal fibrinous material resembling clotted serum, and dermal

* In the temperature range studied, the maximum tissue temperature rise was found to be nearly linearly related to the amount of energy applied and was not perceptibly influenced by the initial temperature. The rise was approximately 5.5° C./cal./sq. cm.

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edema are evident in the lesions on both sides of the ear in each case. Lesions produced by 8.0 seconds exposures to radiant thermal energy are presented in Figures 5 and 6; for the burn in Figure 5 the sweep technique was used, while for that of Figure 6 the exposure was of the shutter type. In general, when the tissue injury was associated with blister formation on both ear surfaces, the injury to either side of an ear, or the apparent damage from ear to ear was nearly the same regardless of the causative agent or the exposure time. Exceptions occurred only in certain cases of fractional second exposures of several tenths of a second where the opposite side showed a less severe injury than did the exposed side.

ET_{50} for Various Exposure Times

Water temperatures required to produce blistering on the exposed sides or on the air-backed opposite side of 50 per cent of the rat ears exposed for times between 0.25 and 300 seconds are given in Table I and Text-figure 1A. With exposures from 1.0 to 300 seconds, no differ-

TABLE I

Median Effective Water Temperatures Required to Produce Blistering

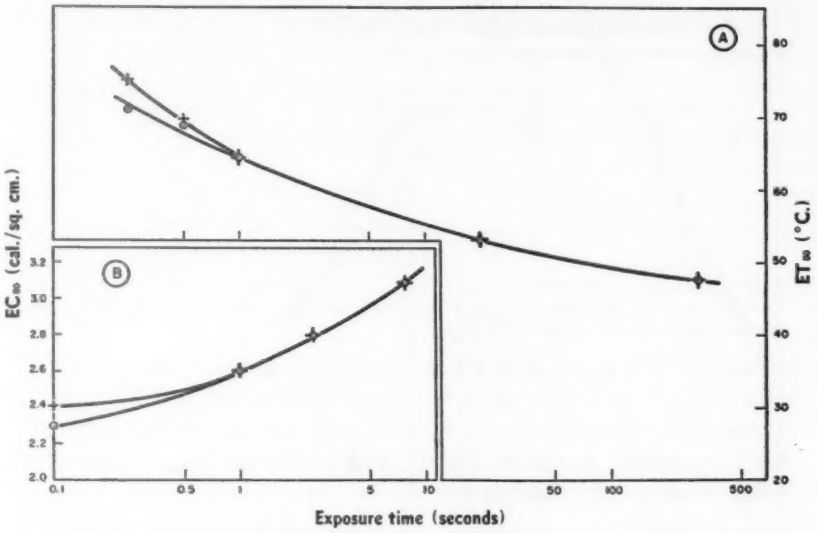
Exposure time	ET_{50}*	
	Exposed side of ear	Opposite side of ear
sec.	°C.	°C.
0.25	71.0±1.1	75.3±1.1
0.50	69.0±0.2	70.0±0.2
1.0	64.4±0.2	64.4±0.2
20	53.0±0.2	53.0±0.2
300	47.6±0.2	47.6±0.2

* Ranges shown with each value are the 95 per cent confidence intervals. The slope, defined as the change in probit for each unit change in the logarithm of the dose, for the 0.25 second burns was about 50; for the remaining burns it was above 100.

ence was found between the ET_{50} for the opposite sides of an ear. The temperature required to produce the effect increased from 47.6 ± 0.2° C. for a 300 second exposure to 64.4 ± 0.2° C. for a 1.0 second exposure. Reduction of the exposure time to 0.5 second increased the required water temperature to 69.0 ± 0.2° C. for the exposed side and to 70.0 ± 0.2° C. for the opposite ear surface. In the 0.25 second exposures, the water temperature required to cause the specified effect on the exposed medial surface was 71.1 ± 1.1° C. For the lateral surface it was 75.3 ± 1.1° C.

To test the influence exerted on the ET_{50} values by an increased

rate of heat loss from the ear, water of 22 to 23° C. was caused to flow against the opposite surface for 10 seconds prior to termination of a 0.5 second exposure and the ET_{50} values were redetermined (Table I). The ET_{50} for the exposed surface was found to be essentially the same as when air of 20 to 25° C. was in contact with the



Text-fig. 1. Comparison between response of a rat's ear to hot water and to radiant thermal energy for various exposure times.

A. The ET_{50} required to produce blistering by heated water. Encircled dots indicate ET_{50} values, in degrees C., for causation of the desired effect on the ear surface directly exposed to the hot water. Plus signs indicate ET_{50} values for the opposite side of the ear.

B. The EC_{50} required to produce blistering by radiant thermal energy. Encircled dots indicate EC_{50} values, in cal./sq.cm., for the exposed ear surface. Plus signs indicate EC_{50} values for the opposite side.

unexposed surface. The ET_{50} for the opposite side of the ear, however, was elevated approximately 10° C.

EC_{50} for Various Exposure Times

The EC_{50}, in cal./sq.cm., for blister formation on either side of the rat ear is given in Table II and Text-figure 1B. At exposure times of 1.0, 2.5, and 8.0 seconds, the EC_{50} values were equal for the two ear surfaces. Increasing the exposure from 1.0 seconds to 8.0 seconds resulted in an increase in EC_{50} from 2.6 ± 0.1 to 3.1 ± 0.1 cal./sq.cm. When the exposure time was shortened to 0.1 second, the EC_{50} for the exposed side was 2.3 ± 0.1 cal./sq.cm., as compared with 2.4 ± 0.1

cal./sq.cm. for the other ear surface. While a significant difference between the EC_{50} for the exposed and opposite sides could not be demonstrated by the Litchfield-Wilcoxon[6] method of probit analysis, it was found that in the 14 ears which developed blisters on only one side, all blisters were on the side directly exposed to radiation. A test

TABLE II

Median Effective Incident Radiant Termal Energy Required to Produce Blistering

Exposure time	EC_{50}*	
	Exposed side of ear	Opposite side of ear
sec.	*cal./sq. cm.*	*cal./sq. cm.*
0.10	2.3±0.1	2.4±0.1
1.0	2.6±0.1	2.6±0.1
2.5	2.8±0.1	2.8±0.1
8.0	3.1±0.1	3.1±0.1

* Ranges shown are the 95 per cent confidence intervals. The slope of the probit regression line was in the range of 14 to 18.

of whether this agrees with a hypothesis of no difference, made by the sign test,[7] *i.e.*, that all 14 blisters occurred on the same side by chance alone, shows the probability to be less than 1 per cent. Thus, although for 0.10 second exposures the difference between EC_{50} values was small, it seems to be significant.

Subcutaneous Temperature Associated with ET_{50} and EC_{50} Values

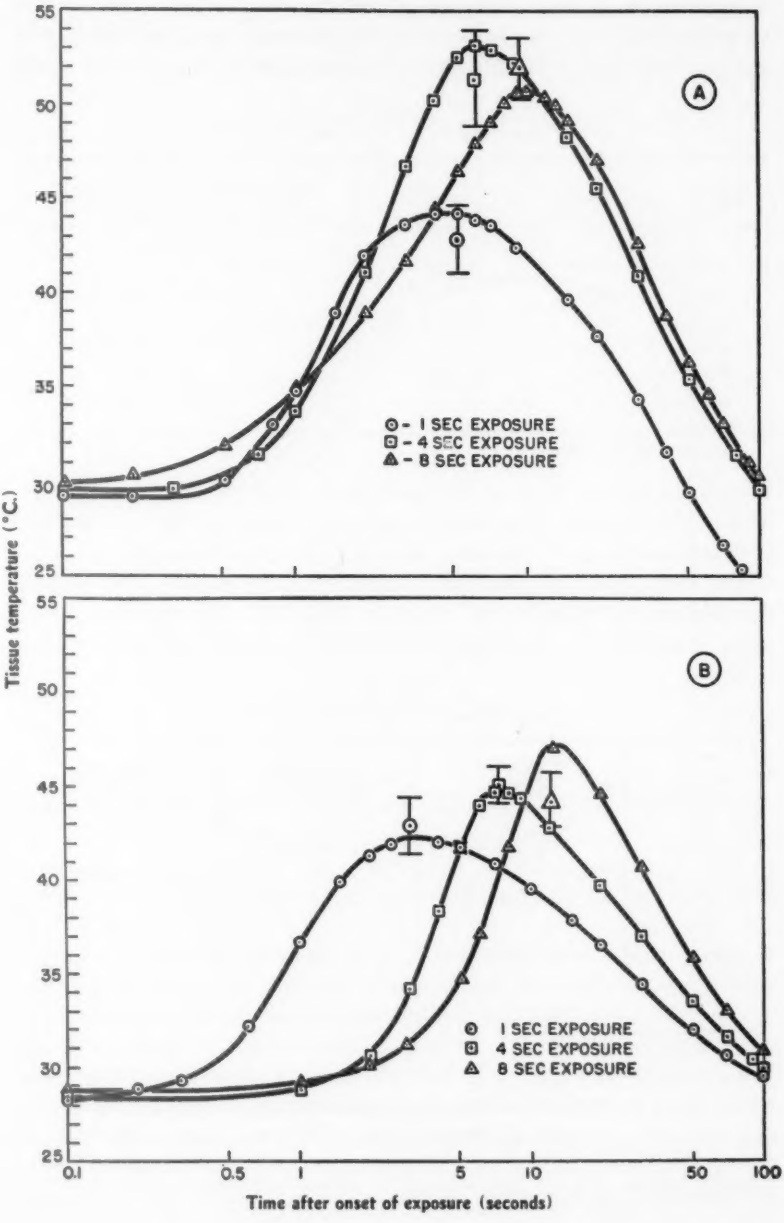
Measurements of tissue temperature were made for the conditions associated with the ET_{50} values of hot water burns. The mean maximum temperatures found for 1.0, 4.0, and 8.0 seconds exposures are 42.9 ± 1.8, 51.4 ± 2.6, and 52.0 ± 1.6° C., as shown in Table III. The mean times required to reach these maxima are 5.0 ± 2.5, 6.1 ± 1.0, and 9.3 ± 0.6 seconds, respectively. Time *vs.* tissue temperature curves, as obtained with the oscillographic recorder, are shown in Text-figure 2A. In this chart the mean maximum temperature and its standard deviation are plotted against mean time to maximum temper-

Text-fig. 2. Curves of average tissue temperature *vs.* time for hot water and radiant thermal burns of 1.0, 4.0, and 8.0 seconds exposures. The mean maximum temperature and its standard deviation are plotted against mean time to maximum temperature for each exposure time.

A. For each exposure time the hot water temperature is equal to the ET_{50} required for blister formation.

B. For each exposure time the radiant energy in cal./sq.cm. is approximately equal to the EC_{50} required for blister formation. The values for maximum temperature have been corrected as described in the text.

Text-figure 2



ature for each exposure time. As a check on the reliability of the temperature values obtained for hot water burns with the subcutaneous thermocouple, in each series of exposures one or more temperature measurements were made in which the exposure was continued until the thermocouple indicated that a constant temperature had been at-

TABLE III

Tissue Temperature Associated with ET_{50}

Exposure time	Water temperature	Mean initial temperature ±stand. dev.	Mean maximum temperature ±stand. dev.	Mean time to maximum ±stand. dev.*
sec.	°C.	°C.	°C.	*sec.*
1.0	64.4	28.9±1.0	42.9±1.8	
1.0	64.4			5.0±2.5
4.0	59.0	27.7±1.9	51.4±2.6	
4.0	59.0			6.0±1.1
8.0	56.0	27.7±1.6	52.0±1.6	
8.0	56.0			9.3±0.6

* Modified linear estimate of σ.

tained. In each such case the final tissue temperature recorded was within 1° C. of the water temperature; for example, tissue in contact with water of 64.4° C. measured 64.0° C. after about 20 seconds.

The mean corrected maximum temperatures for the EC_{50} for radiant thermal energy blistering are given in Table IV. The tissue temperatures found for 1.0, 4.0, and 8.0 seconds exposures are 42.9 ± 1.5,

TABLE IV

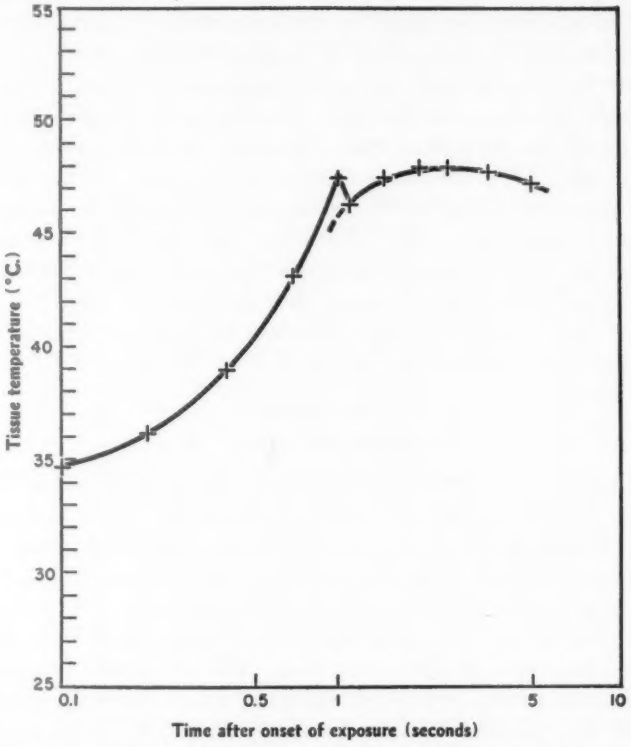
Tissue Temperature Associated with EC_{50}

Exposure time	Mean initial temperature ±stand. dev.	Mean maximum temperature ±stand. dev.	Mean time to maximum ±stand. dev.*
sec.	°C.	°C.	*sec.*
1.0	26.2±1.4	42.9±1.5	
1.0			3.0±0.7
4.0	28.4±0.8	45.1±1.0	
4.0			7.3±0.6
8.0	26.6±1.1	44.1±1.7	
8.0			12.5±1.3

* Modified linear estimate of σ.

45.1 ± 1.0, and 44.1 ± 1.7° C., respectively. For these exposures, the mean times to reach maximum temperature are 3.0 ± 0.7, 7.3 ± 0.6, and 12.5 ± 1.3 seconds, respectively. Time *vs.* tissue temperature curves as obtained with the oscillographic recorder for the radiant burn are shown in Text-figure 2B.

Because of the penetrating nature of radiant thermal energy it was necessary to consider the direct effect of the radiation on the thermocouple. Text-figure 3 shows the temperatures indicated by a thermocouple, placed approximately 0.5 mm. beneath the ear surface, on exposure to carbon arc radiation of 3 to 4 cal./sq.cm./sec. using a



Text-fig. 3. Temperature indicated by a subcutaneous thermocouple for a 1.0 second shuttered radiant thermal exposure of 3 to 4 cal./sq.cm. The initial tissue temperature was 32.2° C.

shuttered 1 second exposure.* The temperature drop which occurs at the end of 1.0 second indicates that a direct effect on the couple does occur but that when the incident radiation is 3 to 4 cal./sq.cm./sec. the drop amounts to only about 1 to 2° C. The effect appears to be over by approximately 0.2 seconds after termination of the radiation. Since in this study the maximum temperatures occurred either after the exposure phase or at least when the incident energy intensity was

* From the temperature drop between 1.0 and 1.1 seconds, the time constant, defined as time to fall to 1/e of total response, for this thermocouple is about 0.07 second.

low, only a slight effect on the measured temperature maxima could have resulted from direct action of radiant energy on the thermocouple. The exposure phase of the time-temperature curves for radiant energy, however, probably was appreciably affected.

The time-temperature curves of Text-figure 2 were used to evaluate the rate of heat loss from the tissue. The time of maximum for each curve was taken as time zero, t_0. The elevation at t_0 above initial ear temperature was divided by the elevation at various times after maximum and the natural logarithm of this ratio was plotted against time. The decay constant, the fraction of temperature elevation lost per second, was then estimated from the nearly straight portion of the resulting plot. The constants found for 1.0, 4.0, and 8.0 seconds radiant burns are 0.030, 0.028, and 0.027, while those for 4.0 and 8.0 seconds hot water burns are 0.032 and 0.032. In the 1.0 second hot water burns, the plot varied considerably from a straight line relation and the constant was not calculated. The uniformity of these constants indicates that, during the period of decreasing temperature, the tissue temperature was essentially independent of the manner of energy application.

Discussion

Blister Formation

Blister formation of the skin is a specific response to injury. It can result from injurious agents of numerous types and has been noted in skins of widely variant types, such as those of the frog, duck, newborn pig, and rabbit.[8] The rat skin blister described in this study appears within minutes following injury and increases slowly to maximum size in a few hours. It is associated with dermal edema and with an increased accumulation of intravenously injected trypan blue in the blister area. As indicated by the high slope of the dose-response data, noted in the footnote to Tables I and II, for a given exposure time, vesication of rat skin occurs over a narrow range of total energy delivery, and, hence, is a useful criterion for comparison of the effects produced by different combinations of thermal energy and exposure time. The present blister should not be confused with the immediately appearing "exploded-blister" which has been described for both white[8] and black[9] skin and which is probably due to generation of steam pressure within the tissue.

Relative Effectiveness of Contact and Radiant Thermal Energy for Tissue Injury

The results of this study show that, using ear skin blistering as the criterion of injury, contact with hot water can produce an effect on tissue similar to that caused by the application of radiant thermal

energy. This is true for either surface of the ear over the range of exposure times studied, namely, 0.25 to 300 seconds, and 0.10 to 8.0 seconds, for hot water and radiant burns, respectively. When the proper combinations of exposure time and water temperature and of exposure time and incident energy intensity are compared, grossly and microscopically, the pathologic changes are indistinguishable. For exposure times of 1.0 second or longer, if the combination is such as to cause blistering on the exposed surface, the tissue response of the opposite skin surface is essentially the same for burns of both types.

Although it was not surprising that both sides of the ear exhibit nearly identical tissue responses to contact and radiant burns, it would seem that with a given single exposure the two sides need not respond to the same degree. Since the temperature gradient across the ear at any time should be less in penetrating radiant burns than in contact burns of equal surface temperature and exposure time, it would be unexpected that contact and radiant burns which produce an equal response on the exposed surface would result in an equal response on the opposite surface. The present observations are in agreement with these considerations. In the case of contact hot water burns of 0.50 and 0.25 second exposure, the ET_{50} for the opposite side is 1.0° C. and 4.3° C., respectively, greater than for the exposed side; the difference found would probably be even larger, if exposure times as short as 0.1 second had been studied. With the radiant burns it was also possible to demonstrate a difference, manifest as an increase in the incident energy required to give the same effect on the opposite side, in the response of the two sides of the ear. The difference was less marked than in the case of contact burns. Thus, for a 0.10 second exposure, the EC_{50}, for radiant burns, was found to be 0.1 cal./sq.cm. higher for the unexposed than for the exposed surface. Since the temperature rise was found to be about 5.5° C./cal./sq.cm., this amount of radiant energy is only sufficient to cause a 0.5° to 0.6° C. rise in the subcutaneous tissue temperature. These observations indicate that, at least for blistering of thin tissues such as the ear of the rat and for exposures of less than 1 second, fall off of severity of injury as a function of depth in the tissue is more rapid for contact hot water burns than for radiant burns. It is probable that the same considerations will be true for thicker tissues.

Effectiveness of Contact and Radiant Thermal Energy as a Function of Exposure Time

As has been found for the production of "white coagulated" burn lesions and of "exploded-blisters,"[10] the EC_{50} for blister formation by radiant energy decreases as the exposure time is shortened from 8.0 to

0.10 seconds. No measurement of energy delivered to the skin was made for hot water burns. However, it is seen, Table III, that the tissue temperature reached for the 1.0 second hot water burns averaged 9.1° C. lower than for the 8.0 seconds exposure, and it seems reasonable to conclude that, to produce a similar effect, less energy was required for the shorter exposure.

Moritz and Henriques[11] found that in human skin, following exposure to heated flowing water, vesication indicates transepidermal necrosis. Their data show that the thresholds for transepidermal necrosis of human and porcine skin are essentially the same. The values, as found by them, for the temperature of hot water necessary to produce threshold damage in porcine skin for exposures of 10, 30, 90, and 300 seconds are approximately 58°, 55°, 52°, and 50° C., respectively. These authors also found that below 44° C. the plot of exposure time-surface temperature, representing threshold temperatures for transepidermal necrosis, tends to become asymptotic to the time axis. The ET_{50} values for blister formation in the skin of rats' ears are 56°, 52°, 50°, and 48° C. for exposures of 10, 30, 90, and 300 seconds, as shown in Text-figure 1A. That the water temperatures necessary to cause vesication are about 2° C. lower in the rat ear than are the temperatures for transepidermal necrosis of porcine skin could be explained by the differences in the criteria used for the threshold effect and/or by the relatively small mass of ear tissue and the consequent reduction in dissipation of energy from the site of the burn. Consistent with the latter explanation are the results of a limited experiment, based on 19 burns, in which circulation of water at 35° C. against the opposite surface of the ear gave an ET_{50} for the exposed side, for a 30 seconds exposure, of 55° to 56° C. as compared with a value of 52° C. when the ear was backed by air at room temperature.

The threshold studied by Moritz and Henriques[11] for porcine and human skin represents the shortest time at a given temperature that caused complete destruction of the epidermis, while the threshold for rat skin is based on blistering in 50 per cent of the burns. However, the very high slope of the log temperature, probit curve from which the ET_{50} was estimated, as noted by a footnote to Table I, indicates that at a given exposure time the change in water temperature required to encompass the 0.5 to 99.5 per cent response interval is small; essentially 1.5 times the 95 per cent confidence interval. It thus appears that the difference between the present threshold values and those of Henriques and Moritz can be easily resolved and that production of blisters in 50 per cent of rats' ears involves a time-tissue temperature episode similar to that required for transepidermal necrosis of porcine skin and, probably, for vesication of human skin.

For 1.0 second exposures the mean maximum temperature within the ear, associated with blistering on both ear surfaces, was the same for hot water and radiant burns; the temperature found was 42.9° C. With both types of burns the temperature values were higher for the 4.0 and 8.0 seconds exposures than for the 1.0 second exposures. The temperatures accompanying the 4.0 and 8.0 seconds hot water burns were 6.3° and 7.9° C. higher than those of the radiant burns. From Text-figure 1 and from the fact that within 20 to 25 seconds the internal ear temperature reaches approximately the temperature of the contacting water, it is apparent that for hot water burns the maximum tissue temperature will decrease with increasing exposure time and that at exposures in excess of about 30 seconds the values will be lower than those found at 8 seconds. It would seem reasonable that for a constant effect it is not necessary that the tissue temperature be elevated to the same extent if treatment is maintained for a longer interval. However, the reason why a maximum tissue temperature of 42.9° C. coupled with a 1.0 second exposure causes the same tissue injury as 52.0° C. and an 8.0 seconds exposure is not clear. It is evident that the tissue injury is related to the duration and elevation of tissue temperature but that the relationship is not a simple one.

Summary

A comparative study of skin burns as produced by contact with hot water or by high intensity radiant thermal energy has been made. Blister formation and subcutaneous tissue temperature of the rat's ear were used as the bases for comparison. The results indicate that, other conditions remaining constant, blister formation occurs within a relatively narrow range of applied energy and is a useful criterion for comparison of effectiveness of various sources of thermal energy. Exposure times from 0.1 to 8.0 seconds for radiant burns and 0.25 to 300 seconds for contact burns were studied.

For these contact and radiant burns the pathologic changes in the skin are similar.

For equal exposures of less than 1.0 second and equal surface injury, the contact burn is probably associated with a more rapid fall off of severity of injury with depth than is the radiant burn.

The median effective water temperature (ET_{50}) and the median effective radiant energy (EC_{50}) for vesication were determined. It was found that at shorter exposure times, a higher water temperature or a greater rate of radiant energy delivery is required to produce a similar effect. For burns of both types it appears that less total energy is required for the shorter exposure times.

Evidence is presented which suggests that, under the described con-

ditions, production of a blister in the skin of the rat's ears involves a time-tissue temperature episode similar to that required for vesication of human skin.

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LEGENDS FOR FIGURES

FIG. 1. Butt welded thermocouple junction. The wire on the left is copper, 0.0006 inches in diameter; that on the right is 60/40 gold-palladium, 0.0013 inches in diameter. × 100.

FIG. 2. A thermocouple (arrow) inserted into a rat's ear. The ear was removed approximately 1 hour after insertion of the thermocouple. At magnification higher than that used here the skin was seen to have slight edema and occasional extravascular erythrocytes in the immediate vicinity of the wire. The lateral surface of the ear is nearer the thermocouple wire. Hematoxylin and eosin stain. × 100.

FIG. 3. Vesication in a rat's ear following 8 seconds exposure to 56.0° C. water. Medial surface is at the bottom. The epidermis is elevated bilaterally and there is fibrinous material between it and the dermis. The epidermal cells have pale-staining, structureless cytoplasm and dark pyknotic nuclei. Moderate dermal and extreme subcutaneous edema is seen bilaterally. The arrows indicate points where epidermal-dermal disruption apparently has been prevented by the presence of hair shafts. Hematoxylin and eosin stain. × 70.

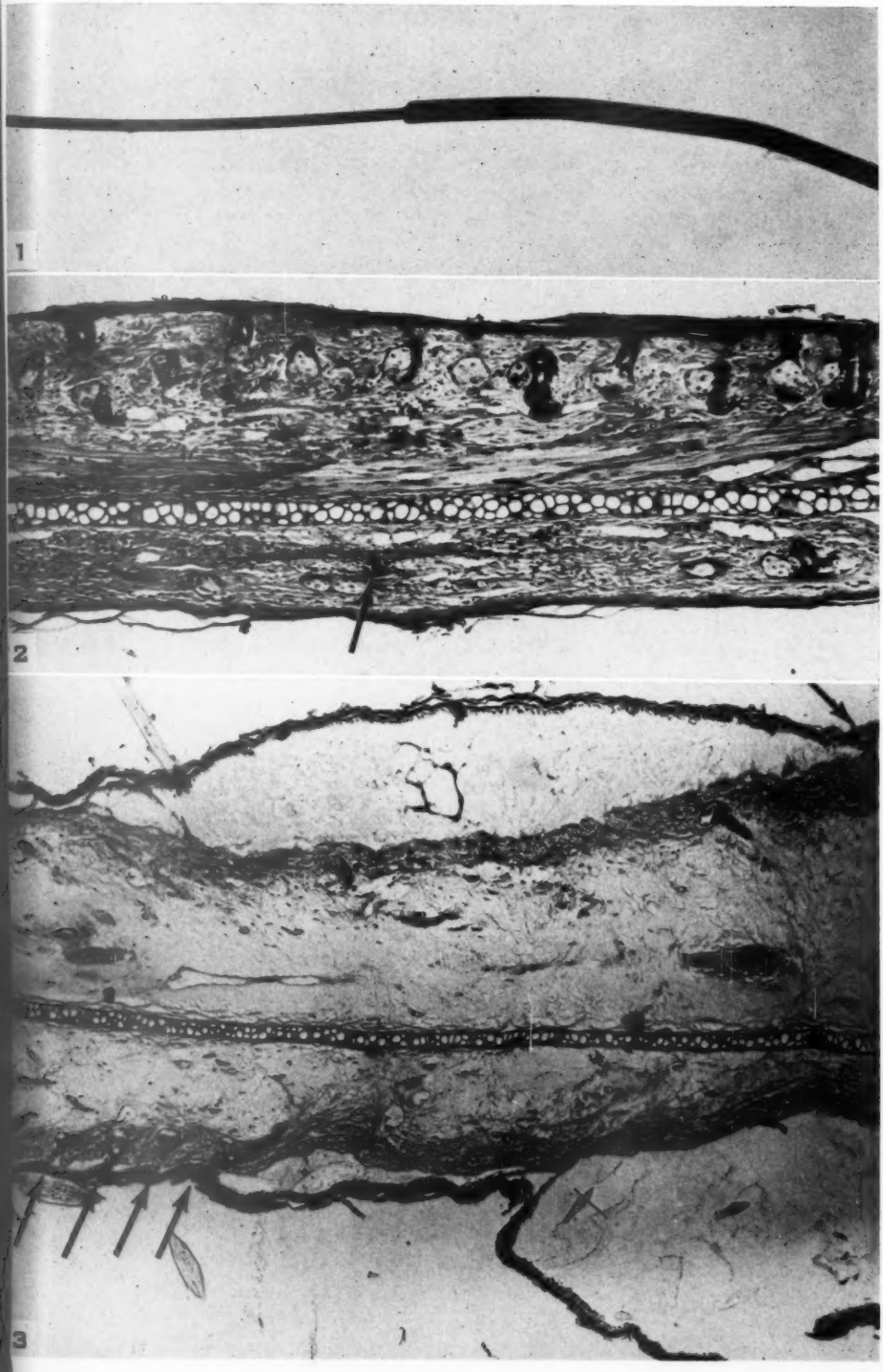
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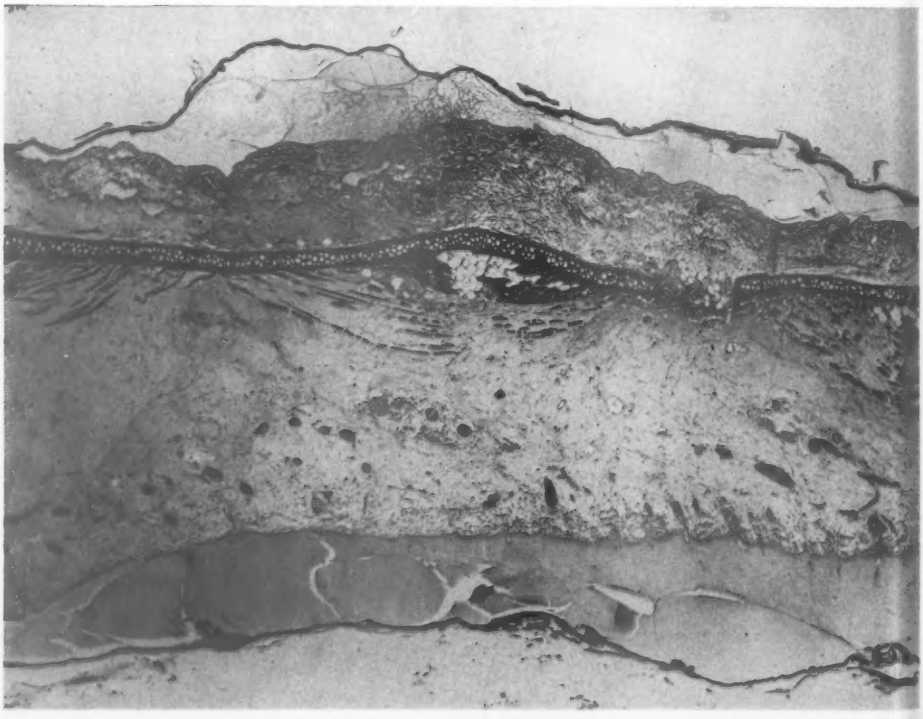
FIG. 4. Vesication in a rat's ear following 4 seconds exposure to 59.0° C. water. Medial surface is at the bottom. Epidermis and fibrinous vesicle contents on either side are similar to those of Figure 3. Marked edema of the exposed, medial side of the ear; slightly less severe edema on the opposite side. Hematoxylin and eosin stain. × 40.

FIG. 5. Vesication in a rat's ear following 8 seconds exposure with sweep technique (3.1 cal./sq.cm.). Medial surface is at the bottom. Epidermis and vesicle contents are similar to those of Figure 3. There is moderate dermal and subcutaneous edema of the medial side. Lateral side shows less severe edema. Medial blister-cap broken during slide preparation. Hematoxylin and eosin stain. × 34.

FIG. 6. Vesication in a rat's ear following 8 seconds exposure with shutter technique (3 to 4 cal./sq.cm.). Medial surface is at the bottom. Epidermis and vesicle contents are similar to those of Figure 3. There is marked edema of the dermis and subcutaneous tissue bilaterally. Hematoxylin and eosin stain. × 60.



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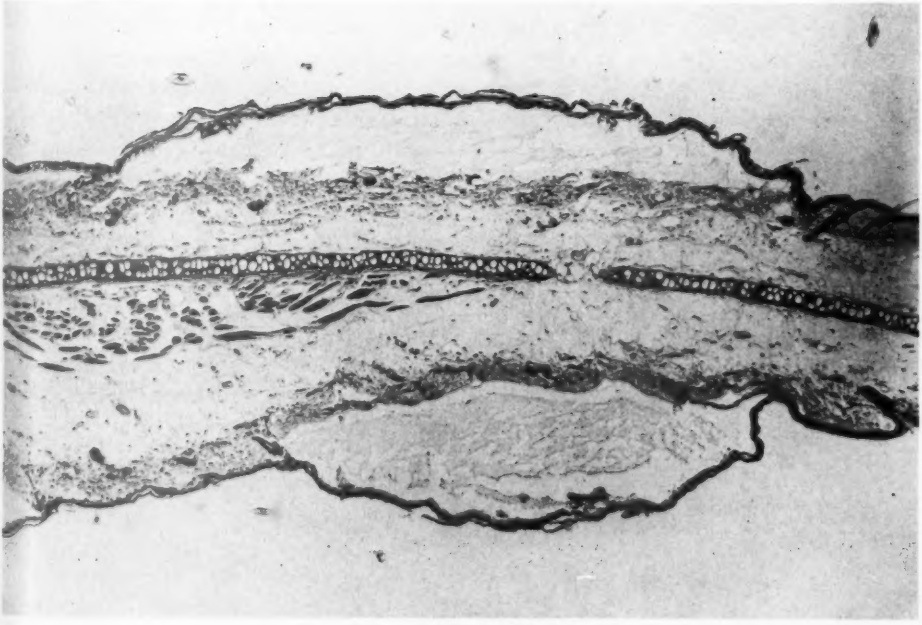

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DIFFUSE CEREBRAL SCLEROSIS (METACHROMATIC LEUKO-ENCEPHALOPATHY) *

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Diffuse sclerosis is the term generally applied to a group of diseases occurring usually in children or young adults and characterized by extensive, approximately symmetric, degenerative changes in the cerebral white matter. The characteristic change is degeneration and loss of myelin sheaths, with lesser and varying degrees of destruction of axons and, more rarely, of glial cells and their fibers. Frequently, a proliferation of astrocytes and fibers occurs in the damaged zones, and this may be so intense as to resemble a neoplasm. The process generally spares the gray matter, although in rare instances, myelin sheaths in this zone are affected, but the neurons are not. In many instances, the most superficial portions of the cerebral white matter, the arcuate fibers, are also spared. In many of these characteristics, the process resembles that seen in multiple sclerosis and in acute disseminated encephalomyelitis. In the latter, focal perivenous zones of demyelinization are characteristic, in contrast to the larger, often confluent plaques of multiple sclerosis. Such perivenous lesions are occasionally noted in the other conditions and further suggest an interrelationship. Until such time as our fundamental knowledge of the etiology and pathogenesis of these diseases is advanced, it is not possible to say with certainty whether these similarities are fundamental or coincidental.

The same considerations apply to the various subdivisions into which many of the cases of diffuse sclerosis may conveniently be placed. These may be variations on the same fundamental theme, or be unrelated except for the fact that varying pathologic processes result in diffuse myelin destruction. The more common forms of the disease were described by Schilder,[1] Baló,[2] Ferraro,[3] Scholz,[4] Pelizaeus,[5] Merzbacher,[6] and Krabbe,[7] and such forms are often designated eponymically. Some of these are familial. Recently, attention has been directed to the presence of metachromatic material in some cases.[8-18]

It is the purpose of this paper to present data on a case of diffuse sclerosis in an infant dying 6 weeks after a 6-weeks-premature birth. Metachromatic material was present, similar to that previously described. The occurrence of an essentially "demyelinating" disease in

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an infant at an age when very little myelin is expected to be present is considered of interest.

Report of Case

The patient, a white male infant, died 6 weeks after birth. The mother had severe diabetes, and had experienced three periods of ketosis and a number of episodes of insulin shock during pregnancy. There were no siblings. Birth occurred 6 weeks prior to term after a short spontaneous delivery from brow presentation. The infant weighed 4 lbs. 5 ozs. at birth and breathed spontaneously after a short pause. Since birth there had been numerous episodes of apnea, intense cyanosis, bradycardia, rigidity, then flaccidity and clonic movements of the extremities, followed by a return of respiration, at first irregular and spasmodic but later normal, and fine tremors of the extremities and head. Neurologic examination between such episodes revealed no specific abnormality. In the general physical examination an enlarged liver was found, and a blowing systolic murmur over the precordium. The infant was considered to be small, with perhaps a disproportionately small head. He expired the day following admission, apparently during a convulsive episode. Weight at that time was 6 lbs.

At necropsy, moderate left ventricular hypertrophy and focal atelectasis of the lungs were noted. The dura showed no apparent abnormality. The brain was of normal size and general appearance. The cerebral hemispheres were symmetric. The gyri were normal in size and contour, the sulci of normal width. The cerebellum and the brain stem appeared somewhat small in proportion to the entire brain. The leptomeninges were thin and transparent. The basal arteries were normal in distribution and of average caliber, and revealed no mural lesions. Sectioning of the cerebrum revealed severe alterations of the subcortical and central white matter of both cerebral hemispheres (Figs. 1 and 2). At the frontal and occipital limits, the white matter appeared opaque, somewhat dry, and granular. The remainder of the central white matter was for the most part completely destroyed, the space being occupied by an irregular multilocular cyst traversed by irregular bands and strands of altered opaque white tissue. The arcuate fibers immediately underlying the cortex were consistently spared and maintained a normal translucent, moist, white appearance. The cortex was of normal thickness. No changes were recognized in the basal ganglia or the thalamus. A large cavum septi pellucidi was present. The lateral ventricles were symmetric and appeared normal in size and shape. The third ventricle, aqueduct, and the fourth ventricle were normal. Section of the cerebellum revealed no changes. In the brain stem the dorsal portion of the medulla was more opaque than the ventral portion. The cord was not examined.

Microscopic sections of the thoracic and abdominal viscera revealed no significant abnormalities. (Frozen sections stained for metachromatic material were not available.) The leptomeninges were somewhat

congested in areas. In some regions, a proliferation of arachnoid cells was noted, some of which contained ingested lipid. The neurons of the cortex appeared approximately normal in number, distribution, and general appearance. The most superficial portion of the subcortical white matter showed no changes. More deeply an increase in the number of glial nuclei was noted (Figs. 3 and 4). Most of these were relatively large, round, and only moderately chromatic. A lesser number were small, round, and deeply staining. A few were elongated. After phosphotungstic acid-hematoxylin staining, a significant increase in glial fibers was not apparent, although the central portions of the white matter stained more darkly than the peripheral zone. Still more deeply, the tissue was replaced by numerous communicating cysts lined by glial tissue and subdivided by irregular septa of similar tissue. The lining tissues consisted of cells with large, pale nuclei as described, small dark nuclei like those of oligodendroglia, microglia, and glial fibers. The number of oligodendroglial nuclei varied, in places appearing to be approximately normal for the age, in places slightly fewer than normal. There were numerous cells with a well defined foamy cytoplasm and a small, dark, often eccentric nucleus resembling the usual fat-filled phagocyte (Figs. 5 and 6). Small quantities of a granular and globular material were found scattered in the tissues, most often bordering a cystic space. This will be described later. Mahon[19] and Weil stains revealed no myelinated fibers in the central or subcortical white matter. The ventricles were lined by ciliated ependymal cells, although in many areas the ependymal lining was lost. Sections of the brain stem showed that the grossly visible distinction between the dorsal and ventral portions was due to the presence of myelin sheaths about the fibers in the dorsal zones and their absence ventrally. Sections of the cerebellum revealed the cortex to be normal. Most of the white matter appeared normal and contained myelinated nerve fibers. A few small focal areas of rarefaction were associated with a proliferation of microglia and astrocytes and with a loss of myelin. The abnormal metachromatic material was not observed in these lesions.

The Metachromatic Material

All of the material studied had been fixed in formalin. In sections embedded in paraffin or celloidin and stained with hematoxylin and eosin, clumps of granular blue material were found in the degenerated white matter (Fig. 5). The individual granules were round or irregular and varied greatly in size, frequently being barely perceptible and occasionally approaching 2 to 3 μ in diameter. They were often grouped in clusters which were round or irregular and varied up to

approximately 40 μ in diameter. Occasionally, an indefinite cloud of such granules was seen. In most instances, there was nothing to suggest that this material was intracytoplasmic. In rare instances, the granules were concentrated in a sharply circumscribed discrete mass as if contained within a phagocytic cell. In no instance was a nucleus or cell membrane discerned with certainty. Most of the material was present in the zone of greatest degeneration at the margins of the cystic cavities. Some was present in the less affected zones of white matter, and very small quantities were present in the white matter immediately beneath the cortex. None was found in the cortex itself. In frozen sections, this material was judged to be present in approximately similar quantity, and was approximately similar in appearance and location.

In frozen sections, the material stained red-violet with toluidine blue, when mounted in an aqueous medium. When such sections were dehydrated in graded alcohols and mounted in a synthetic resin, the metachromasia was lost, the granules staining blue. They were also blue in paraffin-embedded sections stained with toluidine blue. The material was tan with phosphotungstic acid hematoxylin, and blue with the azocarmine technique. The material was positive with periodic acid-leukofuchsin[20] and alloxan-leukofuchsin,[21] but not with the Feulgen technique. The granules were not stained by mucicarmine nor by the Schultz cholesterol technique in paraffin sections. The Gram stain and stains for plasmalogen[20] were negative also. The granules were stained by Nile blue sulfate and by a modification of the acid-hematein technique,[22] although this latter staining persisted after pyridine extraction. The Mahon[19] stain for myelin stained a small fraction of these granules, most being unstained. When paraffin sections were stained by Sudan black (Fig. 6), phosphomolybdic acid,[23] and the acid-fast techniques, the granules were well stained, and, in addition, substances within some of the phagocytic cells were similarly stained although these substances remained unstained by the techniques previously mentioned. The granules were isotropic in polarized light. Ferric salts could be demonstrated within them by acidified ferrocyanide.

When paraffin-embedded sections were dewaxed and immersed in xylol, pyridine, 95 per cent ethyl alcohol, choloroform, ether, or acetone for 3 days at room temperature, or in pyridine or xylol at 80° C. for 2 days, the granular material remained in what was judged to be a quantity equal to that in control sections not subjected to the fat solvents, as indicated by the hematoxylin stains.

When deparaffined sections were hydrated and placed in a solution containing hyaluronidase, 10 viscosity units per ml. (Wyeth) in a 0.02 M phosphate buffer at pH 7.1, no changes were noted in the granular

material after 5 and 24 hours as indicated by hematoxylin staining. A similar negative result was obtained when such sections were immersed in a crude lipase solution ("steapsin"),* 0.5 mg. per ml. for 5 hours, or saturated solution for 1 hour, in a barbital buffer at pH 7.2.

Discussion

General Features

Schilder[1] was possibly the first to segregate a well defined group of cases of what would now be considered diffuse sclerosis from the greatly divergent cases with which they had been confused until that time. In consequence, the term Schilder's disease is often applied to the group as a whole. The group is quite varied in its clinical features, starting within the first year, as in the cases described by Krabbe,[7] Globus and Strauss,[24] and Russell and Tallerman[25]; before the fourth year, as in the cases described by Jervis,[26] Greenfield,[9] and Brain and Greenfield[10]; or later, as in some of the cases described by Schilder,[1] and those of Baló[2] and Scholz.[4] In Mackay's cases,[27] if they were of this nature, the disease started at birth. The disease may progress fairly rapidly, with death in a few weeks or months, or more often in about 1 year, but some patients[4-6] may live for a much longer period. The symptomatology is varied, with mental changes, varied motor and sensory deficits, often blindness, and occasionally convulsions.

The basic pathologic pattern consists of diffuse, broad zones of degeneration of the cerebral and occasionally of the cerebellar white matter, which appear dull and gray, are often slightly granular and retracted, and may be cystic. The process involves the central white matter essentially, often sparing the arcuate fibers beneath the cortex. The gray matter is usually not affected, although in some instances changes like those in the white matter may be noted as limited to the myelinated fibers of the cortex. Microscopically, the involved areas show a severe demyelinization, with lesser destruction of axons, a varying, often intense proliferation of astrocytes, and usually, but not always, an appreciable proliferation of phagocytic cells. In some instances an inflammatory reaction results in an accumulation of lymphocytes in the perivascular spaces. Nearly all of the cases described by Schilder[1] showed such inflammatory changes, and in a narrower sense the term Schilder's disease may be restricted to those with such inflammation. An older interpretation, that those cases with inflammation differ essentially from those which appear only degenerative in character, is not universally accepted at the present time.

Other variations of the basic pathologic pattern were described by

* Obtained from the Fisher Scientific Co., New York, N.Y.

Pelizaeus[5] and Merzbacher,[6] in whose cases there was a peculiar persistence of normally myelinated fibers in the perivascular zones. Scholz's cases[4] were characterized by the presence within some of the phagocytic cells of a lipid staining a pale yellow-red with scarlet red, in contrast to the bright red of neutral fat. In Baló's case[2] there was a peculiar concentric lamellation in the affected zones, whereby rings of preserved myelin alternated with rings of myelin destruction. The cases described by Greenfield[9] and Brain and Greenfield[10] were distinguished in part by a profound loss of the interfascicular oligodendroglia, and by the presence of granules of a metachromatic material in the involved areas. Other cases characterized by the presence of metachromatic material include those described by van Bogaert and Dewulf,[15] Brandberg and Sjövall,[16] Norman,[17] Leslie,[18] and Einarson and Neel.[11-14] Einarson and Neel[12] also described cases to which they referred as the "glioblastomatous-reactive" type, implying a relation to neoplasia. In cases of this type, the course is rapidly progressive and of great severity, so that the patients present clinical features of an expanding brain tumor, an impression which appears to be confirmed by an operative biopsy revealing numerous large and somewhat distorted astrocytes. At necropsy it is evident that in such cases the cells do not infiltrate the adjacent gray matter, as would be expected of neoplastic cells, but are limited to the degenerated white matter and so appear reactive. These authors also included in the "glioblastomatous-reactive" group, cases in which a true neoplasm, glioblastoma multiforme, had resulted in destruction of brain tissue, including the myelin of the white matter. Although they concluded that the neoplastic changes had occurred as part of the process of diffuse sclerosis, many investigators have been reluctant to accept such interpretation.

The Metachromatic Material

The earliest reference to the finding of metachromatic mucoid material in brain tissue was probably that of Alzheimer[28] in describing a case reported by Baroncini. More complete studies were presented by Buscaino,[29] Grynfeltt,[30] Bailey and Schaltenbrand,[31] Ferraro,[32] Grinker and Stevens,[33] and Smith.[34] These reports include descriptions of metachromatic material in otherwise normal brains and in brains showing a great variety of pathologic conditions, in many of which an appreciable demyelinization is not observed. Some have related its appearance to a more or less specific degeneration of the oligodendroglia,[30,31,33] while others have favored a relationship to myelin catabolism.[32,34]

Actually these two theories of the origin of the metachromatic

material are not as divergent as may appear. From the time of their discovery by Hortega, the oligodendroglia have been considered to be related in some way to the formation and maintenance of myelin. Direct evidence for this is meager. It rests in part on the fact that no other function is apparent for these, the most numerous cells in the adult central nervous tissues. Some have reported that these cells contribute to phagocyte formation,[35] an unexpected property for ectodermal cells, but this is disputed by many. These cells are more numerous in the white matter, where they are arranged in a linear fashion along the myelinated nerve fibers. It is known that oligodendroglial cells are present in small numbers in the fetus, increase rapidly after birth, are approximately half as frequent as astrocytes at 2 months of age, are equal in number to astrocytes at 6 months of age, and thereafter approach the adult proportion.[36] In a general fashion, the increase in oligodendroglia tends to parallel the formation of myelin sheaths.[36,37] An almost exact simultaneity was claimed for the appearance of oligodendroglia and myelin sheaths in some of the long fiber tracts.[37] Conversely, the oligodendroglia tend to disappear from demyelinated lesions of varying character. This is true of the plaques of multiple sclerosis, but it is equally true when demyelinization is secondary to a known deleterious factor such as ischemia. It would appear that oligodendroglial cells are sensitive to injury, showing little if any capacity to react, and their disappearance in demyelinated lesions is of limited significance. In the cases described by Brain and Greenfield,[9,10] however, a more specific relationship was suggested by the observation that the oligodendroglia had disappeared from some zones in which demyelinization had not as yet occurred. Lumsden[38,39] suggested the possibility that oligodendroglia are capable of preventing the action of lipolytic enzymes which may hydrolyze myelin lipids, and that their destruction permits these enzymes to act.

Some Chemical Aspects of Myelin Catabolism

The process of demyelinization has been most carefully studied in wallerian degeneration,[40-42] *i.e.*, following the destruction of the axon, the integrity of which is absolutely essential for the maintenance of myelin. These studies have been carried out most frequently in the peripheral nervous system. In such cases there is first a physical fragmentation of the sheaths, with a loss of the normal continuity and the formation of irregular ellipsoids and globules. Only when this is well advanced may chemical changes be demonstrated. The normal myelin sheath is reported to consist of a protein-lipid complex or complexes arranged in concentric layers.[43] The lipids include cholesterol, cerebro-

sides (hydrolyzed to galactose, sphingosine, and fatty acids, often lignoceric or phrenosinic acids), sphingomyelin (hydrolyzed to choline, phosphoric acid, sphingosine, and lignoceric or other acids), probably phosphatidyl serine (hydrolyzed to glycerol, fatty acids, phosphoric acid, and serine) and possibly plasmalogen (hydrolyzed to glycerol, fatty aldehyde, phosphoric acid, and colamine).[40,41] The chemical changes in wallerian degeneration include a decrease in the concentration of these substances and in total lipids, an increase in neutral fats (after a transient drop), the appearance of cholesterol esters, and the release of choline.[40,41] Histochemically,[42] these changes manifest themselves in the appearance of material staining by the Marchi technique (chromation followed by osmic acid) and later by the oil soluble dyes, such as Sudan IV. There is a loss in frozen sections of the normal anisotropism and of the normal positive staining by Baker's acid hematein method for phospholipids[22] and by the periodic acid leukofuchsin technique.

The demyelinization which occurs in the demyelinating disease may or may not be similar. Under these circumstances the axon is often characteristically preserved in contrast to the situation in wallerian degeneration in which axon destruction is the primary factor. A morphologic distinction is apparent in the fact that in wallerian degeneration the demyelinization is total in those fibers in which the axon is destroyed, while in multiple sclerosis, "shadow plaques" of incomplete myelin destruction may be noted. In these areas, morphologically intact myelin sheaths are observed which are stained by myelin methods, but significantly less intensely than normal myelin sheaths. Lumsden[39] suggested that in such cases there is a loss of some of the outer concentric lamellae in which the protein-lipid complexes are arranged, or, alternatively, the water content between lamellae is increased.

The lipids in the demyelinating diseases have been studied by Weil[44] who reported a significant decrease in phospholipids in both the gray and white matter of the brain of 4 patients with multiple sclerosis. Brante,[40] studying 3 cases of diffuse sclerosis, found the lipids of the cortex to be approximately normal, while those of the white matter were decreased, most markedly in respect to the myelin-specific lipids. As a result, the proportion of the various lipids in the white matter approached that in the gray. It is of interest, however, that the white matter of a case of viral encephalitis, in which considerable demyelinization had occurred, showed comparable changes. Roizin[45] performed histochemical studies in one acute and 3 chronic cases of multiple sclerosis, as well as in animals with experimental allergic encephalo-

myelitis. In staining characteristics the lipids resembled those found in demyelinization under other circumstances.

The Significance of the Present Case

The pathologic features of the case presented generally resemble those of other cases of diffuse sclerosis. The process was unusually severe, with a greater than average degree of tissue destruction so that large cystic spaces were noted. The resemblance to swayback, a related disease of lambs, is quite striking because of this feature.[46] The development of cystic spaces in cases of diffuse sclerosis is seen occasionally, and liquefaction has also been described.[47] The astrocytosis and microgliosis are characteristic. The maintenance of oligodendroglial cells in approximately normal number in the less affected zones of the white matter is of considerable interest. It indicates that the mechanism suggested by Brain and Greenfield[10] does not apply to this case. The literature contains numerous reports in which the number of oligodendroglial cells was reported as normal (Einarson and Neel,[11,12] cases 1, 4, and 8; van Bogaert and Dewulf[15]), as only slightly decreased,[17,18] as well as some which emphasized a profound loss of cells (Greenfield[9]; Brain and Greenfield[10]; Einarson and Neel,[18] case 10). Although the oligodendroglial cells may be of fundamental importance in some cases, they are certainly not the sole factor.

The most important feature of this case is the age at which the disease occurred. The infant was born prematurely, after a period of gestation of 34 weeks. His appearance at birth was consistent with this estimate. The disease was apparent very shortly after birth, and resulted in death in 6 weeks. It is fair to assume that the state of maturity of this infant at death was approximately similar to that of the newborn and that the disease process, on clinical grounds, had begun at or before the 34th fetal week. It has been shown repeatedly that the newborn human brain contains comparatively little myelin in the cerebrum, although the cerebellum and brain stem are appreciably myelinated.[36,48] In this connection the term myelin is applied to the substance or substances about the axon which are stained by osmic acid or mordanted hematoxylin as in the various myelin stains. Relatively little myelin is present in the central white matter even at 6 weeks, although a major portion of this area is myelinated at about 4½ months, and the process is almost completed at about 9 months.[36] It is therefore apparent that very little myelin would be expected to be present in the central white matter of this infant at the time of death, and certainly at the time the disease process had started, even

if there had been no pathologic changes. To characterize the disease process as a demyelinating disease in this case seems inappropriate.

Although myelin in the sense described is not significantly present at birth, some of the lipids of which it is composed are present in the white matter in concentrations higher than in the gray. This is true in normal brains without abnormal metachromatic deposits of the type described. Brante[40] suggested that this might indicate that these myelin-specific lipids occur in the axons in higher concentrations than in the cytoplasm of the neurons. An alternative interpretation would be that these substances already are present in intermediate stages of myelin anabolism without as yet having achieved that structure which permits the usual myelin staining. The neutral fat present regularly in the newborn brain[49] has been interpreted[36] as being a by-product of the metabolic changes occurring in myelin formation, although others have considered its presence pathologic.

The abnormal metachromatic material described has been studied in this and in other cases in which it has occurred, because of its obvious relationship to the problem outlined. Its metachromasia, which has been stressed in the literature, did not persist after passage through alcohol. If it had, the presence of acid sulfate groups, as in the mucopolysaccharides, might have been inferred, but not with certainty. The mucicarmine stain was negative. Since it was not alcohol-stable in the present case, the metachromasia suggests merely that the material contains many acid groups of varying types.[20] The periodic acid-leukofuchsin staining suggests the presence of carbohydrate, such as in the galactose of the cerebrosides, a myelin-specific lipid. It may also result from hydroxyamino groupings, such as in the sphingosinol of sphingomyelin and the cerebrosides, and possibly from the double bonds of unsaturated fatty acids which are present in the non-myelin specific lipids as well.[50] The latter is unlikely in view of the negative reaction for plasmalogen.[51] The phosphomolybdic acid stain was claimed to be specific for choline derivatives, this group being the only known substance which renders the acid insoluble.[23] Choline is present in sphingomyelin, a myelin-specific lipid, but also in lecithin, which is present in other structures as well. The staining by the oil soluble dyes, such as Sudan black and Nile blue sulfate, suggests the lipid character of this material. Nile blue sulfate is less significant since it acts also as a simple basic dye. These oil soluble dyes, unlike Sudan IV, are said to favor the staining of the complex lipids, especially phospholipids. The acid hematein method of Baker[22] is considered quite specific for phospholipid if some positively staining proteins are excluded by showing that the material thought to be a phospholipid is soluble in pyridine. In this case the material proved to be insoluble in a varie-

ty of fat solvents, including pyridine. This would be most simply explained by assuming the existence of a complex with protein, for which the alloxan-leukofuchsin staining technique offers more direct evidence.[21] The acid-fast stain, positive in this case, is often ascribed to lipoprotein complexes.

The histochemical observations suggest also that the material is not a single substance, but a mixture of related substances. Very little of it was stained by the usual myelin stains; much of it was stained by hematoxylin or toluidine blue; whereas Sudan black, phosphomolybdic acid, and possibly the acid-fast techniques stained additional material within many of the phagocytes. When the properties of the material in this case are compared with those described for other cases in which similar studies were made, it is found that the properties are not identical in any two cases. With hematoxylin and eosin, or comparable stains, most of the material was basophilic, but in Leslie's case[18] it was not. In some cases the granular material was unstained by Sudan IV, while in others a pink or rose coloration was described. The material was described as sometimes tan, sometimes blue with phosphotungstic acid hematoxylin, while Greenfield and Brain's[9,10] cases contained material of both types. Other differences may be noted from Table I. It may be said, however, that in most cases, as in the one being reported, the histochemical evidence suggested that the normal material was of a complex nature, containing protein, very probably carbohydrate, and a lipid moiety which showed features resembling those lipids characteristic of myelin. A relationship to myelin is strongly suggested, but not proved. Parenthetically, it is to be noted that the tendency to separate a "pure metachromatic" type,[11-14] characterized by the presence of large quantities of isotropic, metachromatic granules which do not stain with mucicarmine and are not accompanied by significant quantities of neutral fat, from a "fatty metachromatic" type, in which the abnormal metachromatic material is often anisotropic, may be mucicarmine positive, and is associated with large deposits of neutral fat, is not entirely applicable to the present case. In this instance, a metachromatic, isotropic material which was not stained by mucicarmine, was associated with considerable quantities of neutral fat. Some have preferred to restrict the term metachromatic leuko-encephalopathy to the "pure metachromatic" cases, which are less common.[10,18]

If a relationship between the metachromatic material and myelin metabolism exists, it cannot reflect simply the chemical changes of myelin destruction, at least in this case, since little or no myelin could have been present to be destroyed. A relationship to myelin formation is much more likely. In this connection, Sperry and Waelsch[52] showed by radioisotope techniques that the lipids of the nervous system are

TABLE I

Some Properties of the Abnormal Material

Procedures or their equivalents*	This case	Leslie[16]	Norman[17]	Greenfield and Brain[9,10]	Einarson and Neel				van Bogaert and Dewulf[15]	Brandberg and Sjövall[16]
					I[11]	IV[11]	VIII[12]	X[12]		
Hematoxylin and eosin	Blue	Red		Blue	Red-brown		Blue	Blue	Slight	
Nissl-aqueous	Violet	Red	Pink	Pink	Violet	Violet			Violet	Orange-yellow
Nissl-alcohol	Blue	Blue	Blue	Blue					Blue	
Phosphotungstic acid hematoxylin	Tan	Blue		Yellow-blue						
Azo-Mallory	Blue	Variable		Blue-red					Blue-red	
Periodic acid Schiff	Red	Red		Red						
Mucicarmine	No	No	No	Yes	No	Yes	No			No
Sudan IV	No	Pink	Pink	No	No		No	No	Rose	Violet
Sudan black	Black	Black		Variable					Gray-black	
Myelin	Black	Black	Negative	Black			No			
Smith-Dietrich-Baker[23]	Black			No					No	Black
Nile blue sulfate	Blue		Blue	Pink					Blue	
Acid fast	Red									
Polarized light	Iso	Iso	Iso	Iso Aniso	Iso		Iso			
Phosphomolybdic acid[24]	Blue									
Solubility in ether	No	No	No	No	No					
Pyridine	No	Yes	Yes							
Choloroform	No	Yes	Part		No					
Alcohol	No	No	No	No	No		No			
Acetone	No	No	No		No					
Xylol	No	No	No		No					
Effect of embedding	No	Decrease	Lost	No						
Location Free in tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
In phagocytes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
In neurons	No	Yes	Yes	Yes	No	No	No	No	No	No
In viscera	No	No	Yes	Yes	No	No	No	No	No	No
Associated neutral fat	Much	Slight	Slight	Slight	Slight	Much	None	Much	None	Slight
Decrease in oligodendroglia	No	Slight	Slight	Loss	No	No	No	Loss	No	Loss
Familial	No	No	Yes	No	No	No	No	No	No	Yes

* This chart was prepared with considerable hesitation in spite of its obvious interest, because of the difficulty in obtaining reliable comparable data. Some of the data are direct quotations from the original, but many items are interpretations by me based on ancillary data. Thus, grouped under the heading of Nissl stains, are the results with cresyl violet, toluidine blue, and thionine. The Smith-Dietrich-Baker methods varied. Under the heading of Sudan IV are grouped the results with this and related substances. Even the hematoxylin and eosin data are, in some cases, interpretations of the results by other methods, such as iron-hematoxylin-van Gieson in the Greenfield and Brain reports.

synthesized *in situ*. They further showed that these lipids are not static, but that there is a continuous replacement of them at a slow rate, approximately 10 per cent a week in the rat. It would follow that even the disappearance of myelin from sites in which it had been deposited need not be interpreted as myelin destruction in an active sense. If no new myelin were being formed, myelin might disappear as a result of its normal catabolism. The possibility exists that the abnormal metachromatic materials seen in adult cases do not represent an abnormal intermediate stage in myelin destruction, but an aberrant intermediate product in myelin synthesis, as is postulated for this infant. It is noteworthy that van Bogaert and Dewulf[15] described metachromatic granules in their cases in portions of the white matter in which the myelin appeared intact.

The thesis that the primary defect lies in myelin formation has been offered by others, notably Brain and Greenfield[9,10] and Norman.[17] Brain and Greenfield suggested that an abnormal formation of myelin renders that substance unstable and easily susceptible to destruction. In the cases described by these authors, metachromatic material was present in the cytoplasm of neurons and in some of the viscera, notably the liver and kidney. In Norman's case, such material was present in the kidney, bile ducts, and a papilloma of the gallbladder. Witte[8] had previously reported the presence of metachromatic material in neurons, white matter, anterior pituitary body, testis, liver, and kidney, while Leslie[18] subsequently described such material in neurons as well as in the white matter. This widespread involvement suggested that there was a more extensive disturbance in lipid metabolism than mere demyelinization. It should be emphasized that some differences in tinctorial properties and solubility were reported among the various metachromatic substances in the individual cases, suggesting that they may not be as closely related as at first seemed apparent. Witte thought the metachromatic material in the neurons was altered lipochrome pigment and van Bogaert and Dewulf[15] also reported that lipochrome may acquire metachromatic properties after formalin fixation. Norman believed that the metachromatic substance in the neurons of his case differed from lipochrome in solubility and in staining properties and represented an abnormal lipid deposit in a disease of the "lipoidosis" group, such as Tay-Sachs' or Niemann-Pick's diseases. Leslie believed that the metachromatic material was produced in the brain and "spilled over" into the blood stream, permitting a secondary deposition in the viscera, an interpretation which avoided the necessity of assuming a generalized abnormality in lipid metabolism.

The pathologic features of this and other cases also serve to direct attention to processes other than that of demyelinization. Some cases,

including this one, showed a profound destruction of many neural tissues, obviously including axons, glial cells and fibers, and even blood vessels. It is to be doubted that the specific destruction of myelin in an area would itself evoke so intense a reaction as to destroy all other neural elements, and in any case evidence of inflammation is often completely lacking. It is suggested that in some cases the primary insult is to the glia and nerve fibers, or both, as well as to the myelin; and that the myelin sheaths, which are so sensitive to injury of almost any sort, may even be only secondarily affected. A similarly intense destructive change may be noted in cases of Devic's disease, which also has been grouped among the demyelinating diseases. Whether similar considerations are applicable to multiple sclerosis, acute disseminated encephalomyelitis, or others of this group is purely conjectural. In multiple sclerosis, the pathologic features are more specifically of the character of a primary demyelinization.

It was noted that the mother of this infant was diabetic and was in poor control during pregnancy, with frequent episodes of ketosis and insulin shock. It is an attractive hypothesis that a relationship may have existed between diabetes in the mother and diffuse sclerosis in the child. However, no direct evidence for this hypothesis exists. We are not aware of other recorded instances of combined parental diabetes and filial diffuse cerebral sclerosis.

Summary

A case of diffuse sclerosis in a 6-weeks premature infant who died 6 weeks after birth is described. Convulsive symptoms were present from birth.

Deposits of a granular metachromatic material were found in the severely degenerated, cystic white matter. This material resembled that described in other cases, and showed properties indicative of a complex glycolipoprotein nature.

An infant of this state of maturity normally would be expected to reveal very few myelinated fibers in the central white matter of the brain. To characterize the disease process as primarily demyelinating seems inappropriate in this case. For the same reason, the granular metachromatic material ought not to be considered an abnormal product of myelin catabolism, but more probably of myelin anabolism.

It is suggested that in some cases of diffuse sclerosis the fundamental pathologic changes result in primary injury to glia and nerve fibers, as well as to myelin, or the latter may even be secondarily affected. Reference is made to the applicability of this concept to the other diseases of the demyelinating group.

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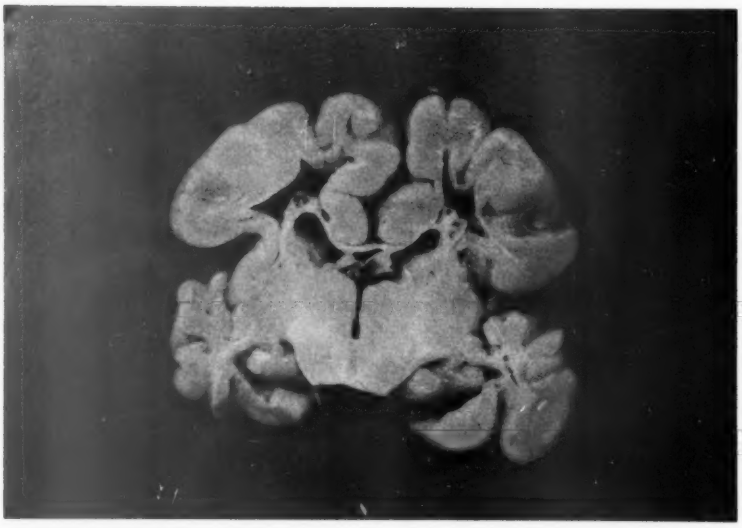
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Coronal section of cerebrum. × 1.

FIG. 2. Coronal section of cerebrum. × 2. The central white matter of frontal and temporal lobes is transformed into multiloculated cysts traversed by bands of glial tissue. The subcortical and arcuate white matter is preserved. The cortex, basal ganglia, and thalamus are normal.

1



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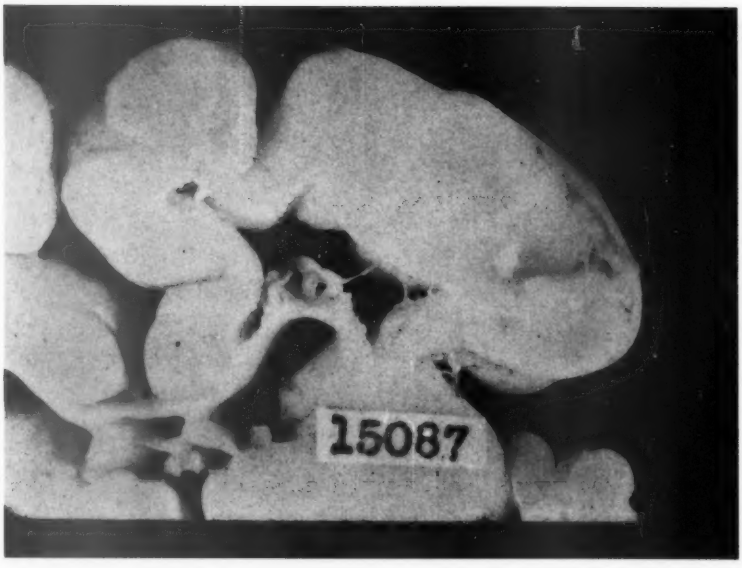
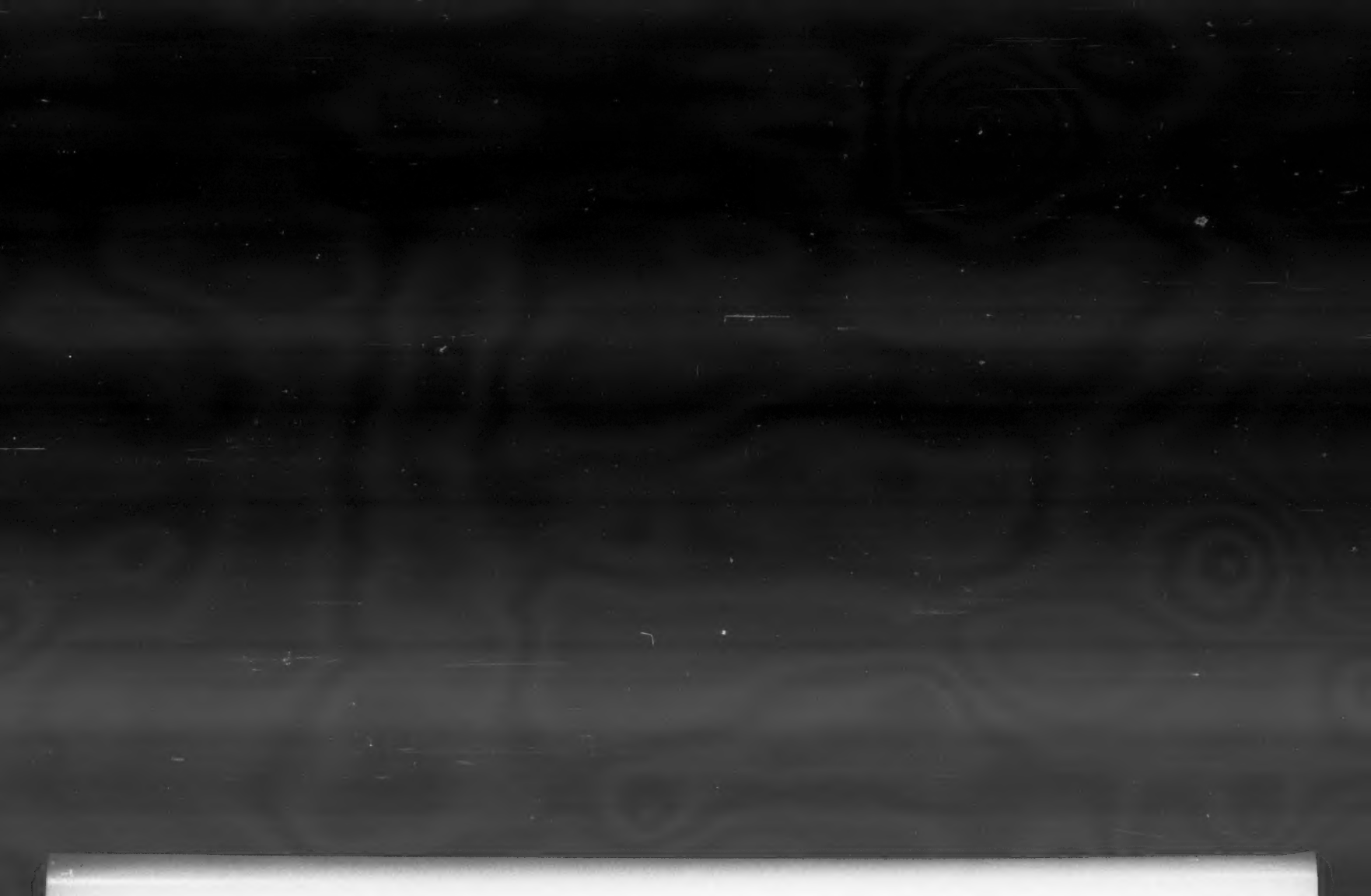


FIG. 3. Cerebrum. Gyrus including cortex on both sides. The arcuate area immediately beneath the cortex is of normal cellularity, while the central strip of deeper white matter is of increased cellularity. Hematoxylin and eosin stain. × 25.

FIG. 4. Subcortical white matter. Nuclei, including those of oligodendroglia, are present in approximately normal proportion. Feulgen technique. × 440.



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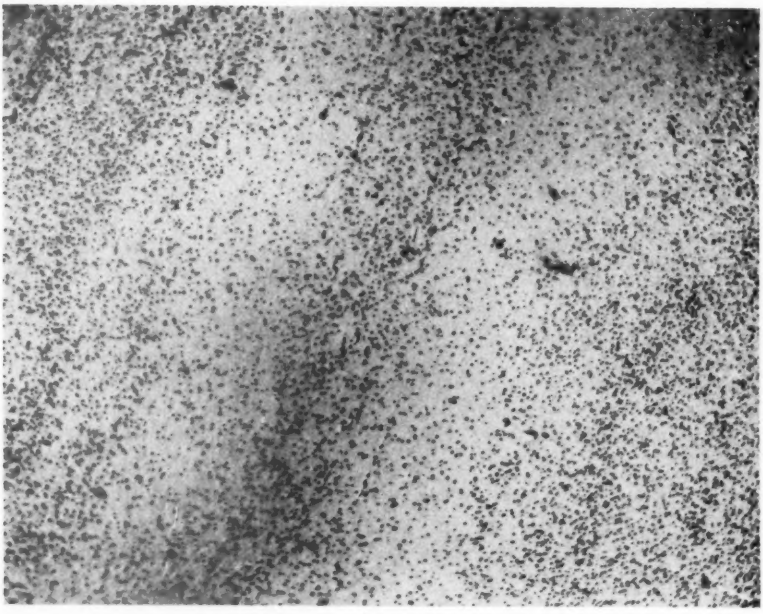

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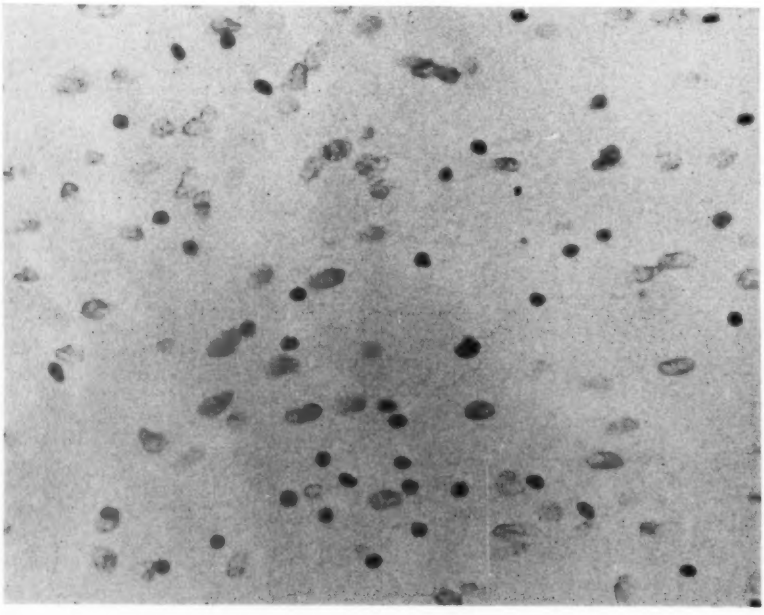
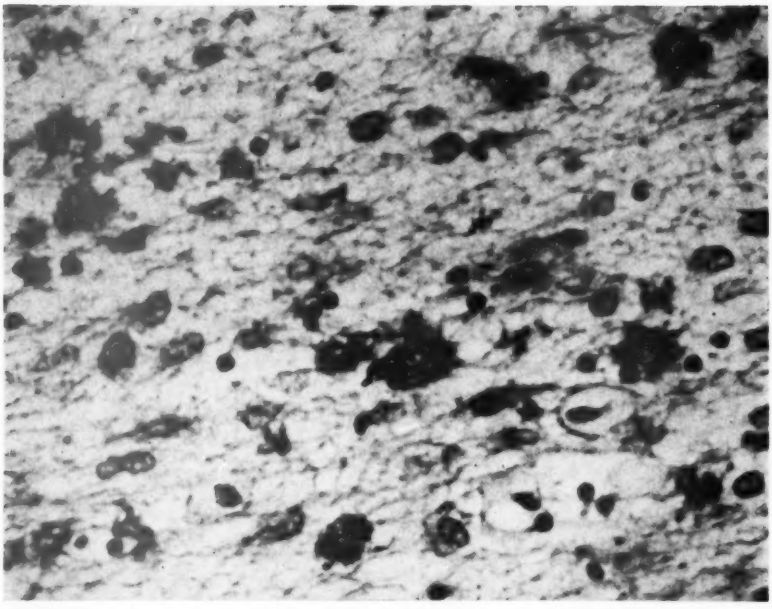


FIG. 5. Central white matter at edge of cyst. Tissue is rarefied and contains phagocytic cells. Clumps of basophilic granular material are present. Hematoxylin and eosin stain. × 440.

FIG. 6. Central white matter at edge of cyst. The granular material is deeply stained, as is some material within some of the phagocytic cells. Paraffin section. Sudan black stain. × 900.

5



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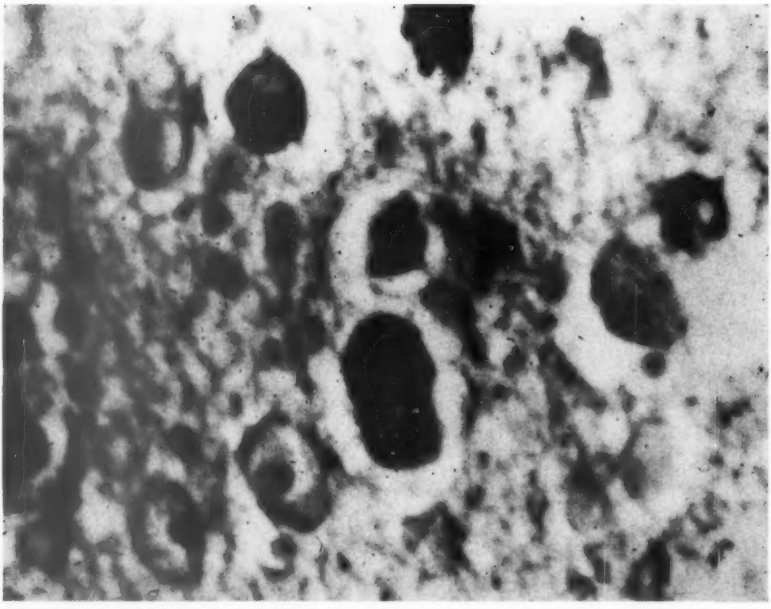

THE RELATION OF THE RENAL LESIONS TO THE CEREBRAL LESIONS IN THE TUBEROUS SCLEROSIS COMPLEX *

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In an earlier paper[1] I submitted, as a hypothesis, that there is a common factor underlying all the extracerebral lesions comprised in the tuberous sclerosis complex. I suggested that this common factor is intrinsic in nature and neural in origin. The cellular tissue in the extracerebral lesions was regarded as akin to the neural tissue of the neurilemma or sheath of Schwann, and the extracerebral lesions were considered to belong to a system disease to which the name neurilemmoblastosis was given. Glioneurilemmoblastosis was suggested as a suitable name when the brain is affected as well as other parts of the body.

In the cases studied for that paper no material from the brain was available for comparison with lesions in other organs, whereas in the present communication comparison is made between lesions in the kidney and lesions in the brain of the same subject.

REPORT OF CASE

In 1928, Dr. W. A. T. Lind of Melbourne sent me portions of a cerebral hemisphere, of a kidney, and of cardiac tissue, from the body of a child with tuberous sclerosis. The following clinical and post-mortem notes were provided by Dr. Lind, who performed the necropsy.

Clinical Notes. The patient, a deafmute idiot and the child of deafmute parents, died after a succession of epileptic fits, practically status epilepticus. The patient did not show adenoma sebaceum during life, but the skin in the region of the adenoma sebaceum area was not the clear skin of a child. There were fibromas on the back.

Post-Mortem Notes. The skin showed very prominent lentigines around the nose and mouth. The brain showed the usual hypertrophic sclerosis, but the "candle-gutterings," although present, were not as prominent as usual. The kidneys showed numerous growths, some of them more deeply situated than is usual in this condition. A careful search revealed nothing else of significance.

NAKED EYE DESCRIPTION OF THE SPECIMENS

Brain. Projecting into the lateral ventricles of the brain were raised bands which, when cut transversely, were found to be 0.5 cm. in diam-

* Received for publication, December 3, 1953.

eter. These bands were not uniform in caliber; they had nodules in them, and thus had a somewhat beaded appearance. Blocks including these bands were fixed in formalin and embedded in paraffin.

Kidney. The renal tumors varied in size from 2 cm. in diameter to microscopic dimensions. They were very numerous, dozens being present in the specimen submitted, which consisted of about one third of a kidney. Some of the tumors were pyramidal with their bases close to the capsule, but many were situated deeper in the renal substance. The largest tumor was partly in the cortex but largely in the medulla and projected into the pelvis. The kidney showed no evidence of fibrosis. Three or four small cysts were observed, approximately 1 to 2 mm. in diameter.

Heart. All of the small piece of heart which was available was embedded.

MICROSCOPIC CHARACTERISTICS

Blocks of cerebral, renal, and cardiac tissue fixed in formalin were embedded in paraffin in 1928. Sections cut from these blocks, in 1928 and more recently, were stained by hematoxylin and eosin, Masson's trichrome stain, and Mallory's phosphotungstic acid hematoxylin stain.

The sections of cardiac tissue showed no changes of special significance. The structure of the cerebral lesions is shown in Figures 1 to 5 and of the renal lesions in Figures 6 to 12.

The Cerebral Lesions

The portions of brain to which special attention was paid were two subependymal lesions. The structure of the first is shown in Figures 1 and 2, and that of the second in Figures 3 to 5.

In Figure 1 a nodule projecting into the lateral ventricle is shown. A flattened layer of ependyma was present on the surface; beneath this was a zone of uninvolved cerebral tissue separating the nodule of hyperplastic gliosis below from the ependymal layer above; deep to the nodule was a zone of uninvolved cerebral tissue. The nodule, though not encapsulated, was fairly well circumscribed.

Figure 2 shows a portion of Figure 1 more highly magnified. The glial hyperplasia with the formation of a dense matted network of fibers, some coarse, some fine, was a striking feature. The fibers appeared to be extensions from the cytoplasm of cells with elongated nuclei, some of which stained deeply, others faintly. Large ill defined cells with faintly stained or barely recognizable nuclei were also a conspicuous feature; cytoplasmic strands from these large cells contributed to the glial network. The fibrillar character of this lesion was

even more striking in corresponding sections stained by Mallory's phosphotungstic acid hematoxylin stain.

Figure 3 shows another nodule projecting into the lateral ventricle. Here again there was a thin ill defined layer of ependyma on the free surface. The ependymal layer rested on an uninvolved layer of cerebral tissue which separated the lesion proper from the lining of the ventricle. This lesion was larger and, though circumscribed, was somewhat less clearly defined than the first nodule. Glial hyperplasia was present, but was less striking than in Figure 1. Two additional features called for comment, namely, the abundant rounded cells in the looser and less dense parts of the nodule, and also the scattered, large, irregular, dark-staining bodies which are believed to consist of calcium deposits.

At a higher magnification (Fig. 4) most of the cells appeared irregularly spherical, with rounded nuclei and fairly dense cytoplasm. A considerable number showed retrograde changes, the nuclei being blurred and the cell outlines ill defined. As is shown in Figure 5, in addition to the spherical cells there were cells with elongated nuclei and cytoplasmic strands, and there appeared to be the beginning of a glial network. It is believed that transitional stages between the two types of cells were present also, though the transition is not very striking in the area illustrated.

The large, irregularly rounded cells, so conspicuous in this second cerebral lesion, and present to a less extent in the first, were looked on as early developmental forms which had arisen from the neural tube. Since gliosis was such a striking feature in these cerebral lesions, and as transitions between these immature cells and the more mature glial cells appeared to be present, the immature cells are regarded, at least in the main, as glioblasts, although it is appreciated that it is impossible from morphologic characteristics alone to be sure of this.

The foregoing description corresponds with that widely recognized to be characteristic of the cerebral lesions of tuberous sclerosis. It now remains to determine the extent, if any, to which similar features are to be found in the renal lesions in this case.

The Renal Lesions

Cysts. Microscopic examination of the kidney revealed scattered small cysts, one of which is shown in Figure 6. This cyst was lined by a conspicuous layer of epithelium. The cystic condition was regarded as a variant of congenital polycystic disease. As is not uncommon in the kidneys of infants, a few glomeruli in this kidney showed

some evidence of sclerosis, but there was no general fibrosis and the cysts were not regarded as mere retention cysts.

Tumors. The most striking feature of the kidney was the presence of numerous tumors. Adipose tissue constituted a large proportion of many of them (Figs. 7 and 8), but in the largest tumor of all (Figs. 9 to 12), adipose tissue, though present (Fig. 12), was inconspicuous, and in most parts absent.

In Figure 7 a lipomatous tumor is shown. It was not encapsulated; indeed, isolated fat cells were evident some distance from the main tumor. Similar appearances were seen where the tumor nodules were composed of rounded or elongated cells that were free from fat. This appearance is believed to be due, not to local infiltration of the renal substance, but to changes occurring *in situ*. It is believed that when small separate foci enlarge, they fuse and add to the bulk of the main tumor, sometimes enmeshing renal structures such as tubules. The adipose tissue did not constitute the whole bulk of the lipomatous tumors; in some situations the fat cells were separated by tissue which was rather structureless in places (Fig. 7), but elsewhere was composed largely of ill defined elongated cells with elongated nuclei (Fig. 8).

The structure of the largest of the renal tumors is seen in Figures 9 to 12. In a few situations elongated cells with elongated nuclei were mixed with rounded cells (Fig. 9), and there seemed to be intermediate stages between these two varieties. The greater part of the growth, however, showed a structure like that illustrated in Figures 10 and 11. It was composed mainly of irregularly rounded cells varying in size but mostly large. Many of these cells were degenerated, but those that were not showed deeply staining nuclei and comparatively dense cytoplasm. Multinucleated forms occurred; in this regard it is recalled that Globus,[2] in his Figure 20, showed giant multinuclear cells in a cerebral tumor associated with tuberous sclerosis of the brain.

Figure 12 shows one of the relatively few areas in this tumor in which fat cells were clearly recognizable. Between the fat cells were other cells with rounded nuclei and deeply staining cytoplasm. These cells, some of which had degenerated, resembled those shown in Figures 10 and 11. The impression gained was that they were not being converted into fat cells, although the evidence was not entirely convincing.

It is suggested that the rounded cells and cells of irregular outline shown in Figures 10, 11, and 12 resemble the rounded cells and cells of irregular outline in Figures 4 and 5. It has already been suggested that the cells in Figures 4 and 5 are immature cells of neural origin derived from the neural tube, and that the great majority of them are precursors of glial cells like those responsible for the hyperplastic

gliosis in the tuberous cerebral nodules (Fig. 2). It is suggested further that the cells in Figures 10 and 11, and the similar ones in Figure 12, are immature cells of neural origin derived from the neural crest. The elongated cells in Figure 9 are regarded as of the same origin but at a later stage of development.

Discussion

The cerebral lesions of the tuberous sclerosis complex have been extensively studied, but less attention has been paid to the renal lesions of this complex, and it is with the renal lesions that the present contribution is specially concerned. I have commented previously on the association between polycystic disease of the kidneys and the renal tumors of the tuberous sclerosis complex.[1] The case recorded in this paper is in keeping with the opinion already expressed that the association is not fortuitous, but that they are linked by a common underlying basic intrinsic factor. I shall, therefore, pay no further attention to this element of renal pathology in the tuberous sclerosis complex.

The renal lesions which are widely recognized as being an important component of the tuberous sclerosis complex are the tumors. To them many names have been given. Critchley and Earl[3] mentioned angiosarcoma, liposarcoma, myosarcoma, myolipoma, fibrolipoma, perithelioma, hypernephroma, and primitive mixed sarcoma. Moolten[4] mentioned angiofibroliposarcoma, fibrolipomyoangioma, fibrolipomyoangiosarcoma, and adenomyosarcoma of the Wilms type. Wilson[5] stated that the renal tumors belong to the category of mixed growths, and that the generic term embryoma may be used to cover all. As a result of this and an earlier study,[1] I have regarded the renal tumors of the tuberous sclerosis complex as variants of a distinctive tumor *sui generis*.

Features of the Renal Tumor of the Tuberous Sclerosis Complex

The renal tumors of the tuberous sclerosis complex are commonly multiple, affecting both kidneys, but occasionally a single tumor only is present. They may vary in size from microscopic dimensions to 10 cm. or more in diameter. Often they cause no clinical manifestations, but occasionally the large ones especially may cause clinical disturbance. To the naked eye they may be white, yellow, or mixtures of the two. The yellow areas are composed of adipose tissue and the white areas of spindle cells, rounded cells, and cells of irregular outline. Multinucleated cells may be present and mitotic figures are scanty or absent. I have not met with metastases, but Wohlwill[6] stated that malignant transformation is not rare. In the cellular areas blood vessels may give an angiomatous appearance. These different structures

may be present in various combinations in different tumors in the same patient, or even in the same tumor. The renal tumor of the tuberous complex differs from the mesodermal mixed tumor of the uterus in that it contains no cartilage and no striped muscle; it differs from the Grawitz tumor and the Wilms tumor in that it contains no epithelial elements.

The term embryoma is commonly used for the Wilms tumor of the kidney, and confusion would arise if this term were used for the renal tumor of the tuberous sclerosis complex.

Epithelial tumors may be present in the kidneys in the tuberous sclerosis complex, for example, the papillary adenomas of Wohlwill's[6] second case; such an epithelial tumor, however, though possibly linked (like polycystic disease) at the basic intrinsic factor level with the renal tumor of the tuberous sclerosis complex, is of an entirely different type.

The Origin and the Interrelation of the Various Components of the Renal Tumor of the Tuberous Sclerosis Complex

The components of the renal tumor of the tuberous sclerosis complex are (a) adipose tissue, (b) tissue composed of rounded cells, elongated cells, and cells of irregular outline, (c) blood vessels.

Adipose Tissue

The renal tumor component which can be identified with confidence is adipose tissue, and this is found in both tumor nodules in which the cellular (non-fatty) component consists of cells which are mainly at an advanced stage of development (Fig. 8) and in nodules in which the cellular (non-fatty) component consists of cells mainly at an early stage of development (Fig. 12). In both varieties of tumor nodules the adipose tissue has, for the most part, a structure such as one would find in ordinary adipose tissue such as subcutaneous adipose tissue, or in a lipoma in that situation.

The fat cells, speaking generally, contain large single drops of fat, and do not present the moruloid appearance of the cells of so-called brown or glandular adipose tissue or tumors arising therein.[7] Further, the adipose tissue in these renal tumors does not resemble that of a liposarcoma. The fat does not seem to be forming in the rounded or elongated cells in those portions of the tumor referred to as cellular as distinct from adipose (though the possibility that some of the immature irregularly rounded cells may form adipose tissue is not altogether excluded).

Let us consider briefly, for purposes of comparison, the overgrowth of adipose tissue found in relation to neurofibroma associated with

macrodactyly.[8,9] Bell and Inglis[8] found overgrowth of fibrous tissue, cartilage, and adipose tissue in a hand, within the area supplied by a neurofibromatous median nerve; these changes are now regarded as local effects due to the influence of intrinsic factor of neurofibromatosis.[9] The influence presumably was exerted after the development of the nervous system, and so was at the level of the neural intrinsic factor rather than at that of the basic intrinsic factor.

Recently Scharenberg[10] has supported the view advanced by Del Río Hortega[11] that the essential neoplastic elements in the peripheral tumors in von Recklinghausen's disease are lemmocytes, and that both schwannoma and neurofibroma (lemmocytoma) originate from displaced elements of the neural crest. In keeping with the view that the essential element of the neurofibroma is the lemmocyte, I now suggest that all the changes (including the lipomatous condition) related to macrodactyly associated with neurofibroma of the nerve supplying the enlarged digit are due to the influence of the lemmocytes, though the exact way in which this influence is exerted is unknown.

Returning from this digression on the adipose tissue overgrowth in neurofibromatosis to the adipose tissue overgrowth in the renal tumors of the tuberous sclerosis complex, we find these two striking resemblances: (1) Neurofibromatosis and tuberous sclerosis are both widely regarded as hereditary diseases of the nervous system; (2) Neurofibromatosis and tuberous sclerosis are both commonly associated with lipomatous tumors.

Since lemmocytes, cells of neural crest origin, are the essential element in the formation of neurofibroma (lemmocytoma) with which lipomatous tumors are so often associated, it is regarded as significant that the lipomatous overgrowth in the renal tumors of the tuberous sclerosis complex are closely associated with tumor cells, of which the advanced forms morphologically resemble the neurilemmal (Schwann) cells of traumatic and spontaneous neurilemmomas (schwannomas), and the immature forms resemble the immature neural cells of the cerebral nodules of tuberous sclerosis as in the case described in this paper.

Tissue Composed of Rounded Cells, Elongated Cells, and Cells of Irregular Outline

In cases described in my earlier paper[1] similar tissue to that now under discussion was conspicuous in the cellular parts of the renal tumors. In the cellular tissue in those tumors the most striking element was the elongated cells which were considered to be akin to the neurilemmal cells of traumatic and spontaneous schwannomas; rounded cells and cells of irregular outline also were present and were looked

upon as more immature tumor elements. In the case reported in the present paper the cellular (non-fatty) portions of the renal tumors contained elongated cells (Figs. 8 and 9), but the rounded cells and cells of irregular outline (Figs. 10, 11, and 12) were more conspicuous. It is suggested that these rounded cells have arisen from the neural crest, and that they are akin to the rounded cells in the cerebral lesions (Figs. 4 and 5) which are believed to have arisen from the neural tube.

The cellular (non-fatty) tissue of the renal tumors of the tuberous sclerosis complex has been interpreted differently by various observers. Norman and Taylor[12] wrote that in their case all tumors examined showed an identical structure, resembling in some respects that of a fibroma; they referred to the tissue as mesenchymal, and they often found small areas of adipose tissue associated with the nodules. This they regarded as dysontogenetic and in no sense neoplastic. They paid no further attention to these areas of adipose tissue except to state that they may well be compared with the cardiac fatty heterotopia present in their case.

In a paper entitled *Plain Muscle Tumours of the Kidney*, Bhende[13] described four cases, in one of which there was also adenoma sebaceum, and in another there were small groups of fat cells amidst the muscle bundles of the renal tumor. He made no further comment concerning the fat cells. Presumably the renal tumors in these two cases, at least, fall into the same category as those reported in the present paper. Bhende regarded the tumors in three of his four cases as myosarcomas, though none of them metastasized.

In my earlier paper[1] I considered that some of the extracerebral lesions of the tuberous sclerosis complex were due to the influence of neural intrinsic factor at the neurilemmal level, whereas now I am inclined to think that, in such lesions as the renal tumors of the complex, neural intrinsic factor may be operating at the neural crest level.

It is widely held that the elongated cells in these renal tumors are involuntary muscle cells, and I admit that on morphologic grounds I cannot exclude this possibility for some of them. Should it prove correct that involuntary muscle cells are present in these renal tumors, then I suggest that perhaps neurilemmoblastic cells, involuntary muscle cells, and fat cells all arise independently from the neural crest. Hörstadius[14] has emphasized that from the neural crest there develops ectomesenchyme which gives rise to connective tissue and cartilage. Though the neural crest gives rise to many more tissues than was formerly believed, I do not know if it gives rise to adipose tissue or to involuntary muscle.

The possibility that the independence of the adipose tissue and the non-fatty cellular tissue of these renal tumors stems back to an even earlier stage of embryonic development, and that these structures are linked by a common intrinsic factor at basic level, needs to be kept in mind, because in the case reported by Norman and Taylor[12] the link between the congenital diverticulum of the heart and the adjoining heterotopic adipose tissue was presumably at the level of the basic intrinsic factor.

Neurofibromatosis and the tuberous sclerosis complex are thought to overlap, especially so far as cutaneous manifestations are concerned; Urbach and Wiedmann,[15] for example, described Pringle's disease (so-called adenoma sebaceum—a component of the tuberous sclerosis complex) and von Recklinghausen's disease in a child 10 years of age. In this regard, and in view of the hypothesis advanced in the present paper, the case described by Foerster and Gagel,[16] under the heading (translated) *Central Diffuse 'Schwannosis' in Recklinghausen's Disease,* is of interest. The patient was a girl who died when 11 years old. Seborrheic eczema, in part having the appearance of adenoma sebaceum, was present on the scalp. There were cutaneous nevi and fibromas and a diffuse new growth of the central nervous system, especially of the basal nuclei, thalamus, and pons. This was composed of elongated cells with fusiform nuclei and with cigarette-shaped nuclei predominating. These tumor cells were regarded as immature precursors of Schwann cells (lemmoblasts).

Scharenberg,[10] supporting Del Río Hortega,[11] stated that there is no morphologic similarity between the elements of Schwann and the blastomatous lemmocytes (of the neurofibroma, *i.e.,* lemmocytoma), though both come from the neural crest. Since Foerster and Gagel[16] mentioned the resemblance of the lesion of the scalp to adenoma sebaceum, and in view of the changes in the brain, it seems possible that in their patient neurofibromatosis and a variant of the tuberous sclerosis complex overlapped. Their description of the nuclei of the tumor cells (lemmoblastic) in the brain as cigarette-shaped is interesting. This description might well be applied to the nuclei of some of the cells of a tumor of the kidney in a patient with adenoma sebaceum whose lesions I discussed in an earlier paper (Inglis,[1] Fig. 2). These cells I regarded as neurilemmoblastic.

Blood Vessels

In my earlier paper[1] I mentioned that sometimes parts of the cellular renal growths in the tuberous sclerosis complex have an angiomatous

appearance, the large vascular channels having thick, often hyaline walls; and that in some parts of these growths the cells of the growth tend to merge in the walls of the large vessels (Inglis,[1] Fig. 20).

Bhende[13] considered that the renal plain-muscle tumors which he described arose from the muscular tissue of arterioles. I am of the opinion that in the cases I described[1] the vascular changes were due to the influence of the cellular neoplastic tissue on the blood vessels. The fact that this cellular tissue is intimately mixed with the fatty tumor tissue, the two being components of the one growth, seems to be against origin from muscle cells in the walls of arterioles. In the case described in the present paper vascular changes are not very striking and do not resemble specially those of angioma.

Conclusions

Evidence is submitted in support of the hypothesis that: (a) The renal tumors of the tuberous sclerosis complex constitute a distinctive tumor *sui generis*. (b) The renal tumors of the tuberous sclerosis complex, like the cerebral nodules, are of neural origin, the cells of the cerebral nodules having arisen from the neural tube, and the essential cells of the renal tumors from the neural crest.

I am grateful to Professor F. R. Magarey and to Dr. F. C. Courtice for granting me the facilities of the Sydney University Department of Pathology and of the Kanematsu Institute of Pathology, respectively. I am indebted to Dr. W. A. T. Lind for providing the specimens on the study of which this paper is based. I wish to thank Mr. S. Woodward-Smith and Mr. K. Clifford for taking the photomicrographs.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Brain. Subependymal nodule projecting into the lateral ventricle. Hematoxylin and eosin stain. × 70.

FIG. 2. Brain. A portion of Figure 1 at a higher magnification. Glial hyperplasia is a striking feature. Hematoxylin and eosin stain. × 275.

FIG. 3. Brain. Another subependymal nodule projecting into the lateral ventricle. Glial hyperplasia is present, but is less striking than in Figure 1. Two additional features call for comment, namely, the abundant rounded cells in the less dense parts of the nodule, and the scattered, large, irregular dark-staining bodies, which are believed to consist of calcium deposits. Hematoxylin and eosin stain. × 35.

FIG. 4. Brain. Part of the loose cellular portion of Figure 3 at a higher magnification. Most of the cells included in this illustration are irregularly rounded, with rounded nuclei and fairly dense cytoplasm. A considerable number of these cells show retrograde changes. A few red blood cells are seen near the center of the figure. It is believed that these irregularly rounded cells, like the similar ones in Figure 5, have arisen from the neural tube. It is suggested that they are akin to the irregularly rounded cells of the renal tumor shown in Figure 11 which may have arisen from the neural crest. Hematoxylin and eosin stain. × 825.

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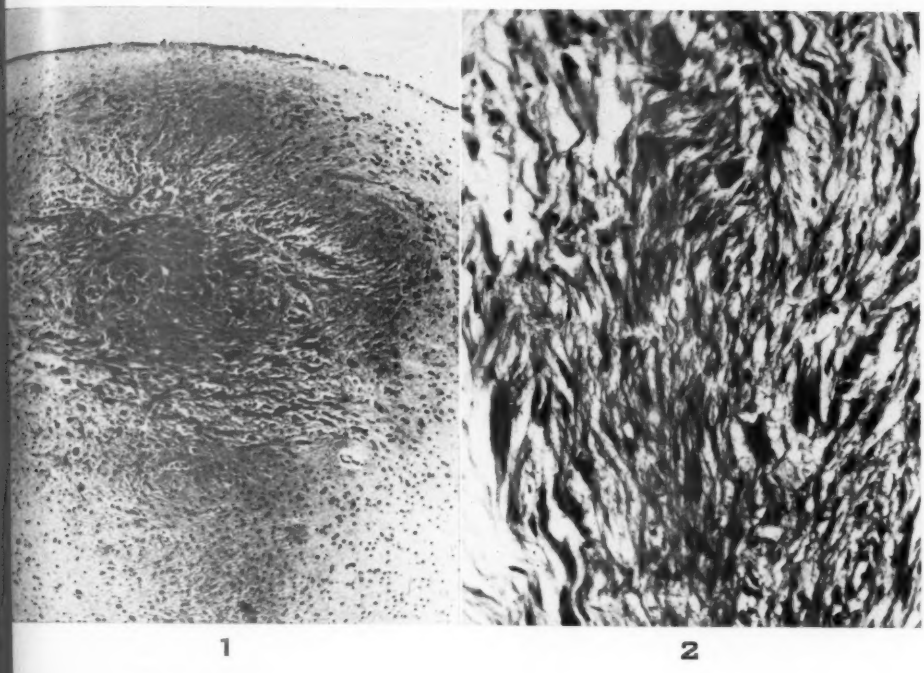

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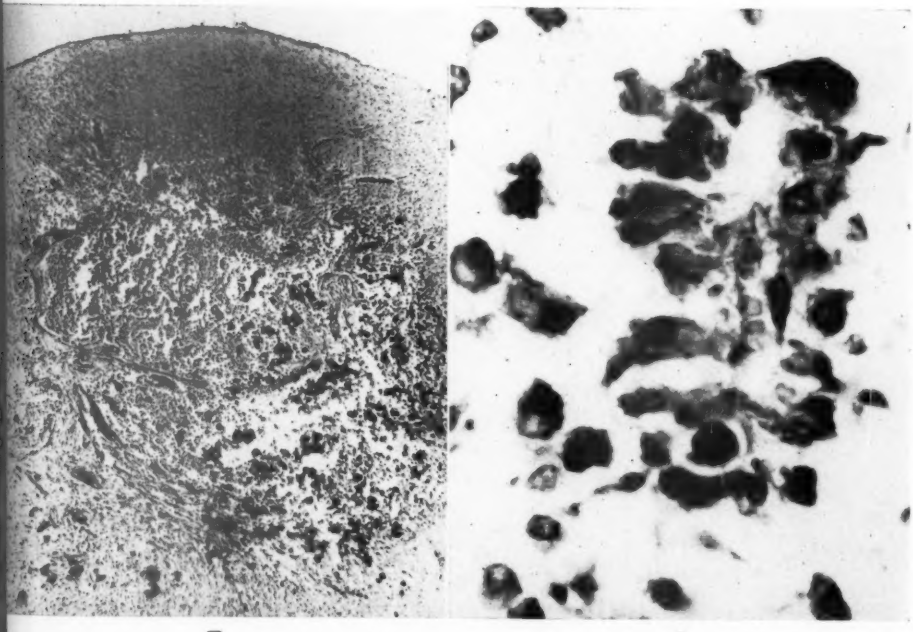

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FIG. 5. Brain. Another part of the loose cellular portion of Figure 3 at a higher magnification. Irregularly rounded cells like those in Figure 4 are present, but in addition there are cells with elongated nuclei and cytoplasmic strands, and there appears to be the beginning of the formation of a glial network. It is believed that transitional stages between the two types of cells are present also, though the transition is not very striking in this illustration. Hematoxylin and eosin stain. × 900.

FIG. 6. Kidney. A cyst is lined by a conspicuous layer of epithelium. The cystic condition is regarded as a variant of polycystic disease. Hematoxylin and eosin stain. × 150.

FIG. 7. Kidney. A lipomatous tumor is seen. It is not encapsulated. In some situations the fat cells are separated by tissue which is rather structureless. Hematoxylin and eosin stain. × 75.

FIG. 8. Kidney. A portion of a lipomatous tumor is shown. The groups of fat cells are separated by dense tissue in which elongated cells with long nuclei are present. Hematoxylin and eosin stain. × 150.

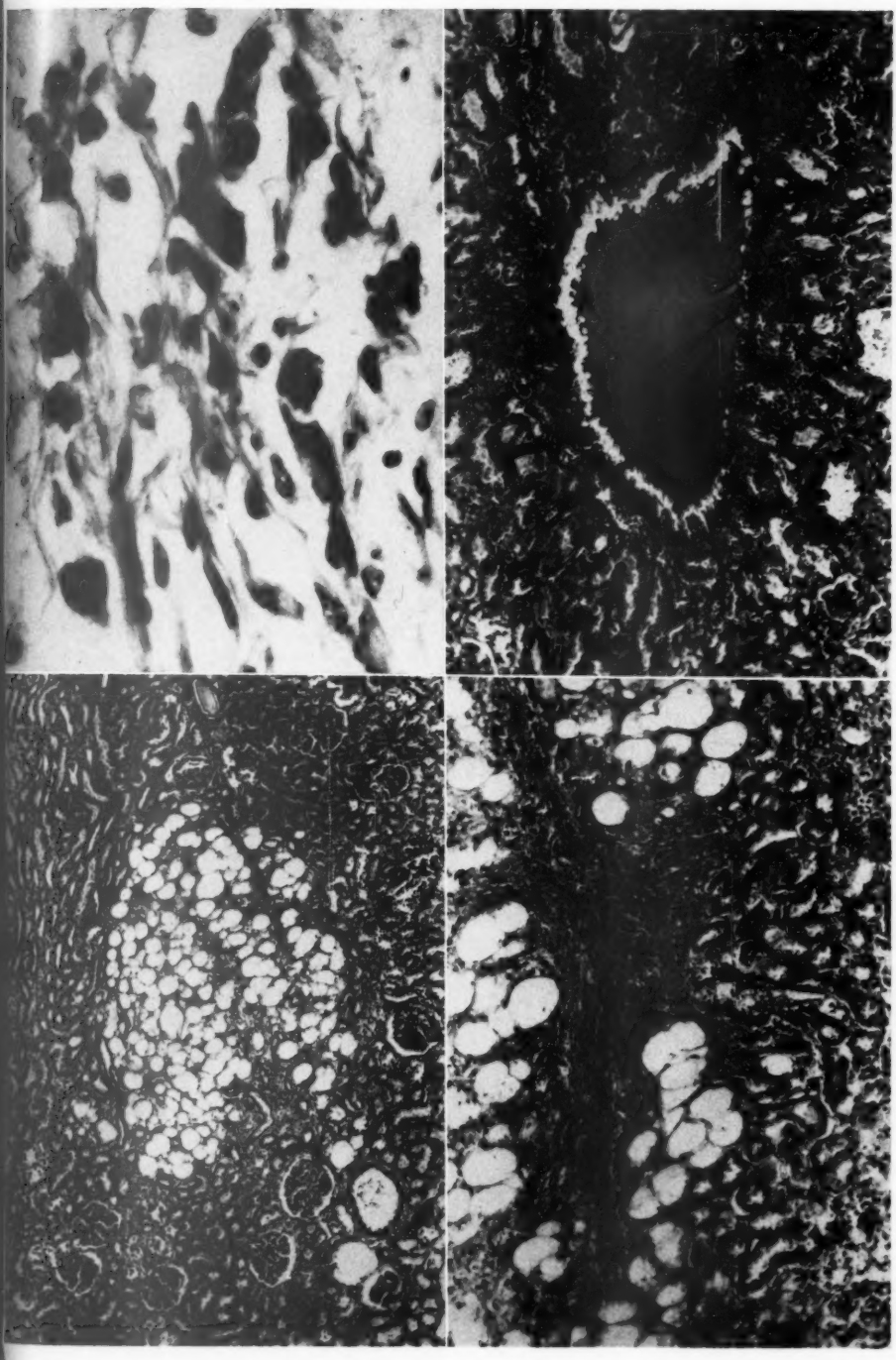

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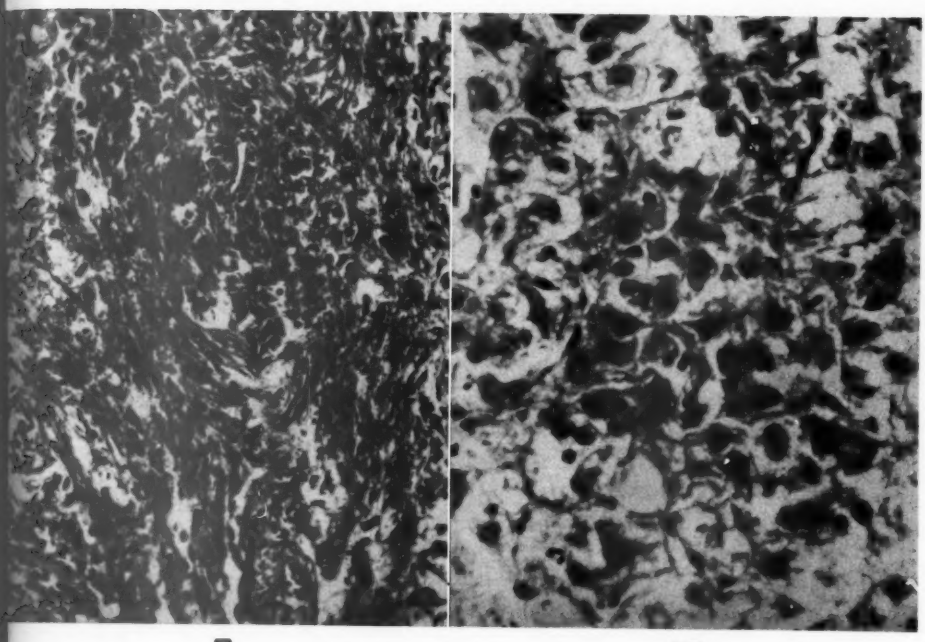
FIG. 9. Kidney. This illustration, like Figures 10 to 12 inclusive, shows the structure of the largest renal tumor. In this figure elongated cells are mixed with rounded cells. Elongated cells were inconspicuous in this tumor. Hematoxylin and eosin stain. × 275.

FIG. 10. Kidney. This shows the structure of the greater part of the largest renal tumor. Irregularly rounded cells predominate; they have deeply staining nuclei and comparatively dense cytoplasm, but many of them have degenerated. Hematoxylin and eosin stain. × 550.

FIG. 11. Kidney. This shows much the same appearances as Figure 10, but the large size of some of the irregularly rounded cells, the dense cytoplasm, the deeply stained nuclei (several of which are sometimes present in the one cell), and the retrograde changes, present a more striking picture. It is suggested that these cells arise from the neural crest, and that they are akin to the large, irregularly rounded cells in Figures 4 and 5, which are believed to have arisen from the neural tube. Hematoxylin and eosin stain. × 825.

FIG. 12. Kidney. This shows one of the relatively few areas in the largest renal tumor in which fat cells are clearly recognizable. Between the fat cells there are cells with rounded nuclei and deeply staining cytoplasm. These cells, some of which have degenerated, resemble those shown in Figures 10 and 11. The impression gained is that they are not themselves being converted into fat cells, although the evidence is not entirely convincing. Hematoxylin and eosin stain. × 550.



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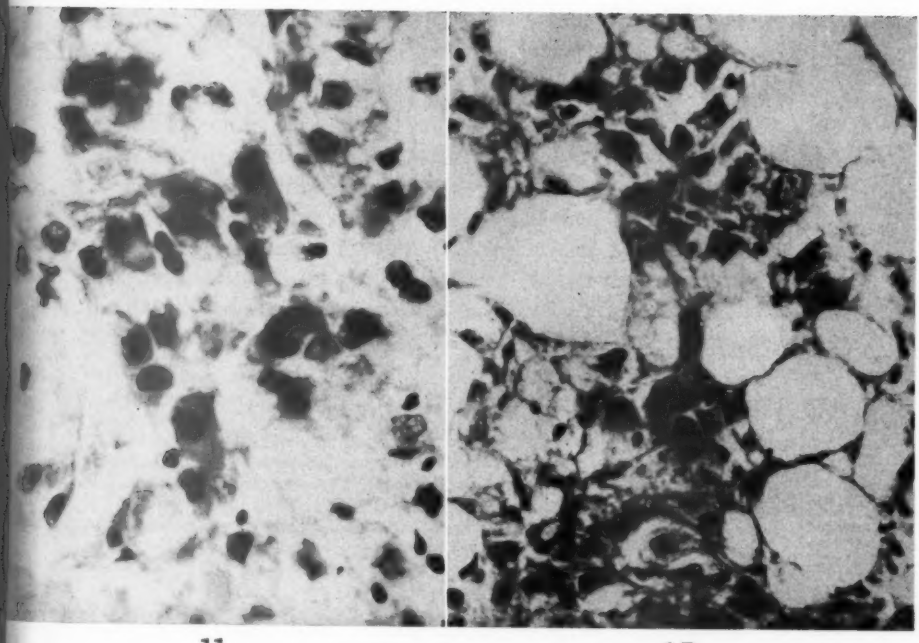

11 12

INFLUENCE OF BLOOD LIPID LEVELS ON INFLAMMATORY RESPONSE IN LUNG AND MUSCLE *

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In 1949 attention was drawn to a form of chronic fibrosing interstitial pneumonitis characterized by unusual deposits of lipid within macrophages.[1] Similar lesions occur focally in association with chronic lung abscess, bronchiectasis, tuberculosis, and sometimes behind neoplastic bronchial obstruction, but in the 10 cases which were described the findings could not be related to any of these conditions. The clinical histories in most instances suggested that the patients had experienced a mild pulmonary infection which was projected into a chronic phase, sometimes in the presence of antibiotics. No etiologic agent was found. It seemed reasonably certain that the lipid material was of endogenous origin. The occurrence of abnormal lipid deposition in pulmonary tissue has been recognized for many years but the significance of the lesions and their relationship to abnormal lipid deposits in other tissues have not been determined.[2]

This report concerns observations on the experimental production of similar pulmonary lesions in rabbits, and particularly on the influence of high serum lipid levels upon the nature of inflammatory reactions. Additional observations concern the inflammatory reactions involving muscles made ischemic by occlusion of the major blood supply in normal and hypercholesterolemic animals.

MATERIALS AND METHODS

Two groups of adult albino male and female rabbits were used. One group received the stock laboratory diet (Purina rabbit pellets) and the other group received a similar diet to which was added 10 per cent corn oil and 1 per cent cholesterol. The cholesterol† was dissolved in the warmed oil and then mixed thoroughly with the pellets. This diet was fed in quantities to yield about 0.5 gm. of cholesterol per animal per day. It was tolerated well and produced a lipemic serum with total cholesterol levels ranging from 1500 to 3400 mg. per 100 ml. in about 2 months.

On these two groups of animals three experimental procedures were

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† Supplied by Armour & Company, Chicago, Illinois.

carried out (Table I). Six lipemic animals and 8 normal animals received intratracheal inoculations of a saline suspension of *Pasteurella pseudotuberculosis*. Another series of 10 lipemic animals and 9 normal animals received intratracheal inoculations of a saline suspension of *Klebsiella pneumoniae* recently isolated from a fatal case of Friedländer's pneumonia. The inoculations were made after ether anesthetization and exposure of the trachea through a small incision. The

TABLE I

Experimental Data on Hypercholesterolemic and Normocholesterolemic Rabbits

Series	Group		Total
	Hypercholesterolemic	Normocholesterolemic	
Friedländer's infection (*Klebsiella pneumoniae*) (Intratracheal instillation)	10 animals (4 hrs. to 40 days)	9 animals (4 hrs. to 47 days)	19
Pasteurella pseudotuberculosis (Intratracheal instillation)	6 animals (2 to 47 days)	8 animals (2 to 47 days)	14
Ischemic muscle (Tibialis anticus)	11 animals (2 to 13 days)	7 animals (2 to 6 days)	18
Total	27	24	51

control and experimental animals were injected with aliquots of the same suspension. In a third series of 11 lipemic and 7 normal animals the major blood supply to the tibialis anticus muscle was ligated under ether anesthesia and aseptic technique. All animals were then continued on their respective diets until they were sacrificed or died. The necropsy material was fixed in 10 per cent neutralized formalin. Frozen sections were stained with Sudan IV and paraffin sections from adjacent tissue were stained with hematoxylin and eosin.

RESULTS

PULMONARY INFECTIONS

Hypercholesterolemic Animals

There was wide variation in the extent of the inflammatory process in the animals infected with the Friedländer organism and also in those infected with *Past. pseudotuberculosis*, but without exception the lesions in these groups of animals were far more extensive than in any of the control animals. The extent of the inflammatory reaction could not be correlated with the duration of infection prior to death. Five of 6 animals died after *Past. pseudotuberculosis* infection while only one of 8 animals died after *K. pneumoniae* infection. Grossly there was whitish yellow consolidation, usually more marked posteriorly, which varied from a lobular distribution to massive solidification of almost

the entire lung. The involved areas were airless and there was no expressable exudate. The intervening parenchyma appeared normal.

Friedländer's Infection. The lungs of animals infected with the Friedländer organism and sacrificed 4 hours later showed a few small foci of neutrophils in the alveoli. Fine droplets of lipid were present in the lumina of the capillaries and were concentrated in some of the endothelial cells of the capillaries; occasionally small groups of lipophages lay free in the airspaces. After 23 hours a severe, diffuse, acute pneumonitis had developed, characterized by edema, many neutrophils, and a few monocytes. Fine lipid droplets were found in the lumina and endothelial cells of the capillaries, and in the edema fluid of the airspaces. Alveolar macrophages contained lipid, but not neutrophils. The lung parenchyma of animals sacrificed on the second day showed accumulations of macrophages in the alveolar septa with neutrophils in the airspaces. A moderate amount of birefringent, sudanophilic material had accumulated in the cytoplasm of the macrophages. Thereafter (3, 4, 6, and 7 days) the alveolar septa and airspaces were packed with macrophages containing large quantities of lipid. The alveolar septal cells were swollen and filled with droplets of lipid (Figs. 1 and 2). Relatively few neutrophils remained, though there was focal acute bronchitis. The animals sacrificed at 9 and 40 days showed a few neutrophils and eosinophils in the septa and occasionally in alveoli. Focal accumulations of lipophages were present in the airspaces. All animals in this series showed arterial atheromas with accumulation of lipophages in the intima in large plaques or circumferentially. At times the capillary endothelial cells in the areas of inflammation were swollen and contained lipid.

Pseudotuberculosis Infection. Within 2 days the animals which were infected with *Past. pseudotuberculosis* had developed an acute bronchitis and pneumonitis with small areas of necrosis. After 4 days many lipophages were found in the exudate surrounding the abscesses, and lipid droplets were present in the capillary endothelial cells, pleural mesothelium, and to a slight extent in the respiratory epithelium of the bronchi. The lungs of animals dying or sacrificed after a longer interval still showed acute pneumonitis with abscess formation, and in the intervening parenchyma there were edema and swelling of the septal cells with macrophages and neutrophils in the swollen alveolar walls (Fig. 3). Many sudanophilic droplets were present in macrophages and in the endothelial cells of the alveolar capillaries. Lipid was more plentiful in macrophages lying in the airspaces. In the later stages of the infection areas of fibrosis had developed in addition to acute necrotizing pneumonitis. The abscesses were surrounded by many lipophages, lymphocytes, and plasma cells. The areas of fibrosis contained

isolated epithelized alveoli, the lining cells containing lipid. These isolated airspaces contained many free lipophages. In addition there were fusiform fat-laden cells trapped in the areas of fibrosis. Lipid was still present in the capillary endothelial cells in the areas of reaction. These animals showed irregular arterial atheromas which were more severe in areas of inflammation than elsewhere.

Normocholesterolemic Animals

Grossly, the lungs of control animals that received identical intratracheal inoculations showed only small focal areas of involvement and in no instance was there massive consolidation such as occurred in the lipemic animals. Many of the lesions were identifiable only after fixation and sectioning.

Friedländer's Infection. The lungs of the rabbit sacrificed 4 hours after inoculation with Friedländer's organism showed a few focal collections of neutrophils in the alveoli. Fat stains were negative. After 23 hours there were areas of hemorrhage with many neutrophils and a few monocytes in the exudate; again no lipid droplets were present. Animals of this series subsequently developed an interstitial pneumonitis with areas of collapse and a variable involvement of the bronchi in the inflammatory process. There was no tissue destruction and the interstitial reaction seemed to be spreading centrifugally from the bronchi and blood vessels. In general, the alveolar septa were swollen by a great accumulation of macrophages with variable numbers of lymphocytes, neutrophils, and eosinophils. Alveolar epithelization had developed (Fig. 4). In some areas macrophages filled the airspaces. Very occasionally there were small foci of acute pneumonitis with neutrophils, and minimal edema and fibrin deposition. Fat stains of these lesions showed only a few sudanophilic, birefringent granules in a small number of the macrophages.

Pseudotuberculosis Infection. In animals infected with *Past. pseudotuberculosis* a focal pneumonitis had developed. The exudate within the airspaces was composed predominantly of lymphocytes and monocytes with a few eosinophils and plasma cells. At times the monocytes were concentrated near the center of the focus of inflammation, simulating a tubercle. At the periphery of each focus there was an interstitial reaction with inflammatory cells infiltrating the alveolar septa. Epithelization of the septal cells had occurred in the diseased areas. In less involved areas there was an interstitial pneumonitis around the bronchi and large blood vessels (Fig. 5). The parenchyma between the foci of pneumonitis showed no change. Fat stains demonstrated that all the inflammatory foci contained a variable number of sudanophilic, birefringent crystals (cholesterol) deposited in the cytoplasm of the

macrophages. The larger, more fully developed granulomas contained a greater quantity of lipid.

DEVASCULARIZATION OF MUSCLE

The major vascular supply to the tibialis anticus muscles of 13 lipemic and 7 normal rabbits was interrupted under aseptic conditions. The muscles were excised at intervals from 2 to 13 days after the procedure. Only 13 of 23 muscles so treated in the lipemic animals and 6 of 11 muscles in the normal animals became ischemic and developed grossly recognizable lesions. At first only the major artery and vein were ligated and a number of muscles escaped serious injury because of collateral circulation. Later the sheath surrounding each muscle was stripped away, and under these conditions almost every muscle showed rather advanced, though variable, ischemia and consequent degeneration. The affected muscles appeared tensely swollen, often hemorrhagic, sometimes frankly necrotic. The cut surface usually showed an irregular line of demarcation between ischemic and vascularized portions of the muscle. The cut surface of the devascularized muscles from lipemic animals was not grossly different from that of the muscles of the normal control animals except for slightly more yellow coloration.

Hypercholesterolemic Animals

Muscle ischemia in rabbits on a high-cholesterol diet resulted in acute necrosis with an infiltration by neutrophils and proliferation of endomysial fibroblasts and macrophages. In more fully developed lesions the necrotic muscle was replaced almost entirely by proliferating cells (Fig. 6). The cytoplasm of these cells contained many lipid granules (Fig. 7). In addition, sudanophilic material had accumulated in the endothelial cells of the capillaries running into the areas of proliferating cells and even in the cytoplasm of endomysial cells surrounding viable muscle fibers.

Normocholesterolemic Animals

In stock-fed rabbits, muscle ischemia led to a focal or massive necrosis of muscle fibers. This change was accompanied by a neutrophilic infiltration and, within a few days, active proliferation of endomysial fibroblasts (Fig. 8). These cells did not contain cytoplasmic lipid granules, except in a few tiny foci.

SUMMARY OF MICROSCOPIC FINDINGS

Animals fed a high-cholesterol diet and infected intratracheally with Friedländer's bacilli showed initially a diffuse acute pneumonitis fol-

lowed by an interstitial reaction, characterized primarily by large numbers of macrophages in the alveolar walls and by alveolar epithelization with apparent desquamation of the septal cells into the airspaces. Large quantities of lipid were found in all these cells regardless of their position within the lung parenchyma.

In the lungs of animals with hypercholesterolemia, pseudotuberculosis produced an acute pneumonitis with large areas of necrosis. The zone of reaction surrounding the abscesses contained many macrophages filled with lipid granules. In this zone the swollen septal cells also contained lipid granules, and sudanophilic material was present also in the endothelial cells of the capillaries and pleural mesothelial cells.

Friedländer's bacilli introduced into the lungs of rabbits on a stock diet produced a focal acute pneumonitis in the first few hours and subsequently an interstitial pneumonitis without tissue destruction. The exudate consisted of lymphocytes, neutrophils, and eosinophils and, in particular, a large accumulation of macrophages in the swollen alveolar walls. There was focal migration of the macrophages into the airspaces. Fat stains demonstrated only a few lipid granules in a small number of macrophages.

The strain of pseudotuberculosis used in this experiment produced focal granulomas in the lungs of rabbits on a stock diet. The cellular exudate was composed of lymphocytes and monocytes with a few eosinophils and plasma cells. The reaction spread into the parenchyma by interstitial infiltration from these inflammatory foci. The monocytes contained a variable, but not impressive, number of birefringent lipid granules. The mononuclear cells in the larger lesions contained lipid in the greater concentration.

Ischemia in the muscles of rabbits resulted in acute necrosis of the fibers with a neutrophilic infiltration and subsequent proliferation of endomysial fibroblasts. In hypercholesterolemic animals there was pronounced accumulation of lipid in the proliferating endomysial cells, whereas in stock-fed animals very few lipid granules had accumulated.

Discussion

The experimental production of pneumonitis accompanied by intrapulmonary deposition of large quantities of fat and cholesterol may help to explain some of the observations on a similar pneumonitis in man. It is a broad step from the hypercholesterolemic rabbit to man, but the almost identical lesions that are found in both lend support to the belief that the basic mechanisms are the same. The fact that qualitatively similar lesions were produced in some animals maintained on a stock diet gives assurance that the lesions in the lipemic animals dif-

fered only quantitatively. Hypercholesterolemia appeared to exert a pronounced effect upon the inflammatory process in the infected lungs, as the inflammatory reaction and mortality were far more severe in the test animals. However, the deposition of lipid within the macrophages may simply augment the reaction to bacterial infection by acting as a foreign substance.

In these experiments the lipid deposits in the lungs and ischemic muscles were hematogenous in origin. Cholesterol deposition within chronically inflamed tissue is common and is generally considered to accompany cellular necrosis. Gross, Brown, and Hatch[3] suggested that the deposition of endogenous lipid within pulmonary tissue resulted from nutritive derangement in detached macrophages leading to fatty degeneration and necrosis. They believed that this mechanism accounts for large lipid deposits as successive generations of macrophages pass through the cycle. The present experiments, however, indicate that activated septal cells become heavily laden with lipid while still in contact with the alveolar walls. The observation of lipid in capillary endothelium in the inflammatory areas also contradicts their explanation for the intracellular position of fatty material. The disruption of the colloidal lipid system could occur intracellularly after passage of fat and cholesterol across the cell membrane, or could occur in the extracellular position with subsequent phagocytosis. The finding of lipid droplets in the edema fluid favors the latter hypothesis.

The accelerated formation of atheromatous arterial plaques in the foci of pneumonitis and the finding of lipid in the capillary endothelium of infected lung and ischemic muscle of hypercholesterolemic animals may be significant. Numerous reports illustrate the experimental production of lipid deposition in blood vessels following trauma, necrosis, and ischemia.[4] The evidence suggests that local factors in infection or ischemia hasten the development of vascular lesions and that the physicochemical phenomena may be the same as those which, under more usual circumstances, result in the gradual development of atherosclerosis.

Intrapulmonary deposits of large quantities of fat and cholesterol have been observed in humans, rabbits, and rats, and have been produced by infection with various bacteria and particulate antimony trioxide.[3] Muscle ischemia resulted in fat and cholesterol deposition within macrophages mobilized in the usual process of removing devitalized tissue.

The variety of experimental procedures that have led to lipid deposition in tissue indicates that non-infectious physicochemical phenomena are responsible and that the problem is not one of general lipid metabolism, except as conditioned by high lipid levels. In all

likelihood the factors concern not only the lipid, but also the associated proteins responsible for stability of the blood and tissue colloids. It is possible that a mild stimulus over a prolonged period can result in the development of xanthomatous lesions in a variety of conditions regardless of blood lipid levels. Specific local etiologic agents may be incriminated in such diseases as the lipidoses now considered to be "metabolic" in nature by many investigators.

Summary and Conclusions

Klebsiella pneumoniae and *Pasteurella pseudotuberculosis* in the presence of high blood lipid levels produced a pneumonitis in rabbits characterized by deposition of large quantities of cholesterol and fat in the inflammatory areas. The pathologic lesions closely resembled "cholesterol" or "endogenous lipid" pneumonia previously described in humans, and were similar to several other types of xanthomatous lesions. In the presence of normal blood lipid levels, fat and cholesterol appeared also in chronically inflamed areas. Similar phenomena occurred in ischemic muscle, suggesting that the process was dependent upon local physicochemical conditions.

The bacterial cultures were kindly supplied by Dr. Lawrence Kunz and Dr. Louis Dienes, Department of Bacteriology, Massachusetts General Hospital, Boston.

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Legends for Figures

Fig. 1. Photomicrograph of the lung of a hyperlipemic rabbit sacrificed 6 days after intratracheal instillation with *Klebsiella pneumoniae*. The walls of the airspaces are swollen by an accumulation of macrophages. Hematoxylin and eosin stain. × 125.

Fig. 2. Photomicrograph of the lung of a hyperlipemic rabbit sacrificed 7 days after intratracheal instillation with *K. pneumoniae*. The walls of the airspaces contain many macrophages filled with fat granules (black). Sudan IV stain. × 125.

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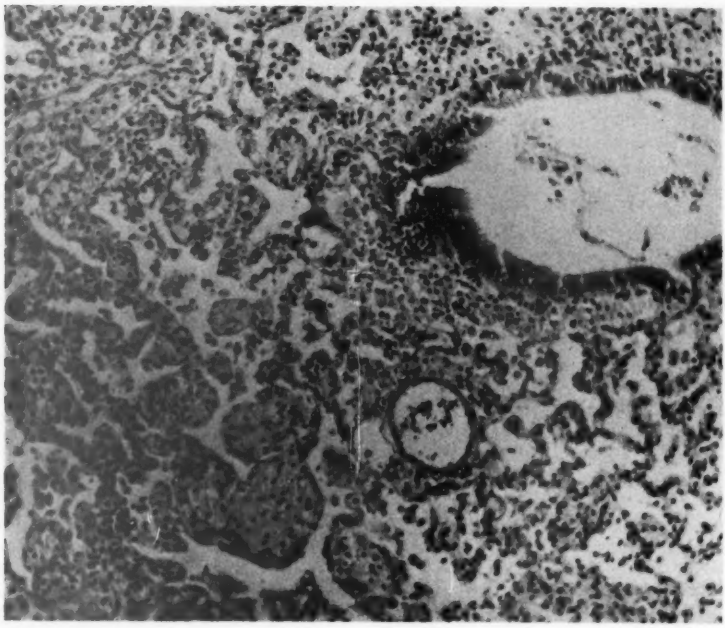

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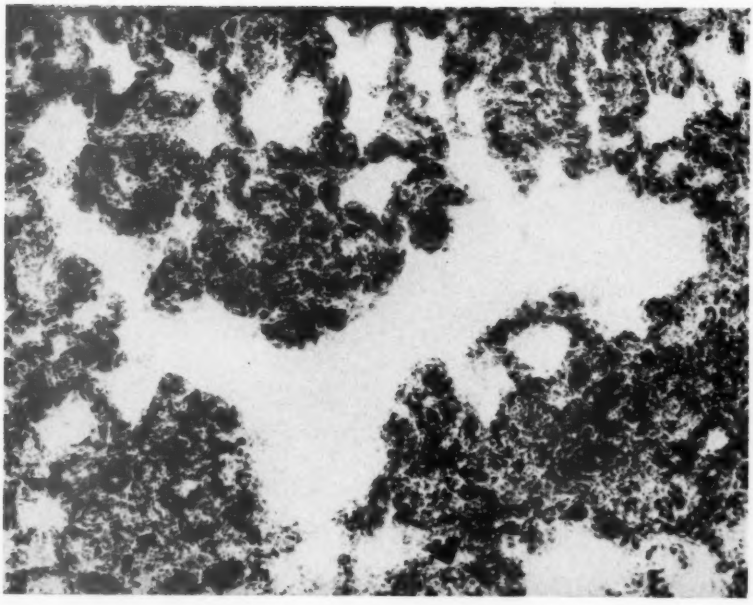
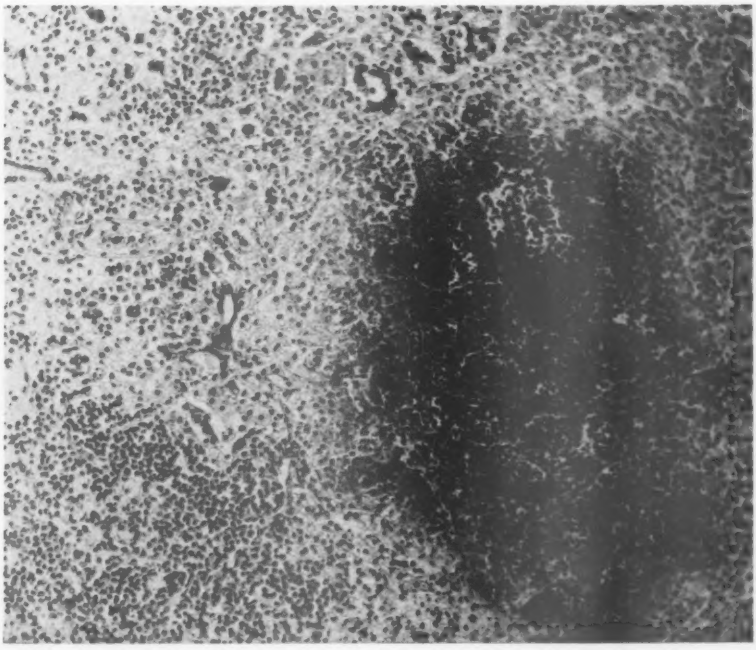


FIG. 3. Photomicrograph of the lung of a hyperlipemic rabbit sacrificed 22 days after intratracheal instillation of *Pasteurella pseudotuberculosis*. Abscess formation is shown, with surrounding acute and chronic pneumonitis. The macrophages of the exudate contained many lipid granules. Hematoxylin and eosin stain. × 125.

FIG. 4. Photomicrograph of the lung of a rabbit on stock diet sacrificed 9 days after intratracheal instillation of *K. pneumoniae*. There is severe chronic pneumonitis, the exudate consisting predominantly of macrophages and lymphocytes; alveolar epithelization is prominent. Only a few macrophages contained sudanophilic granules. Hematoxylin and eosin stain. × 125.

FIG. 5. Photomicrograph of the lung of a rabbit on a stock diet, sacrificed 22 days after intratracheal instillation of *Past. pseudotuberculosis*. A region of interstitial pneumonitis surrounds the bronchus. The exudate is composed of macrophages and lymphocytes. A few macrophages contained a small number of sudanophilic granules. Hematoxylin and eosin stain. × 125.



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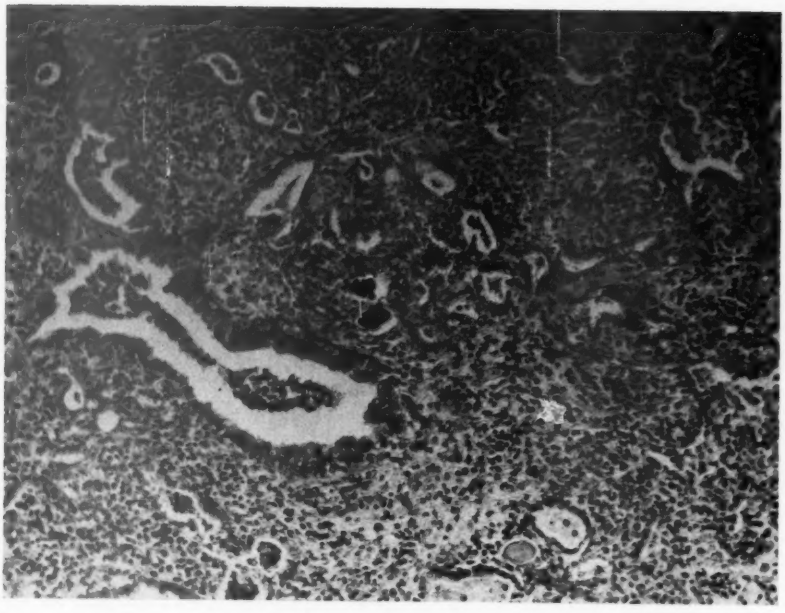

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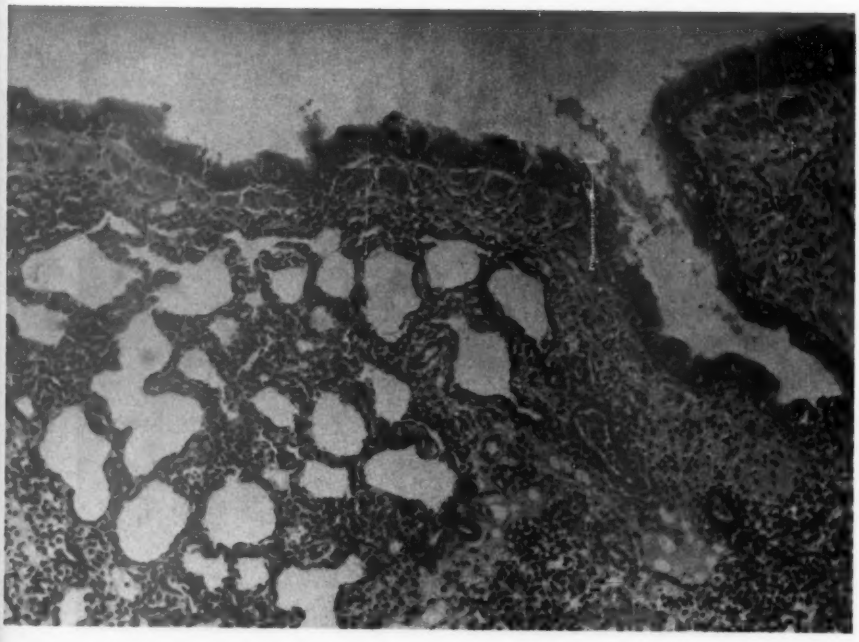
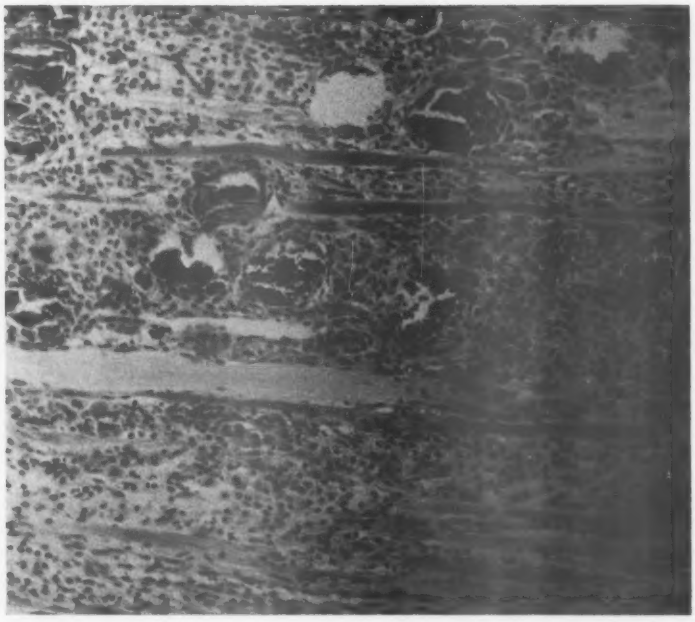


FIG. 6. Photomicrograph of the anterior tibial muscle of a hyperlipemic rabbit sacrificed 7 days after devascularization of the muscle. A few necrotic fibers remain. There is marked proliferation of endomysial fibroblasts and macrophages. There are elongated masses of sarcoplasm with multiple sarcolemmal nuclei. Hematoxylin and eosin stain. × 125.

FIG. 7. Photomicrograph of the same muscle as in Figure 6. The endomysial fibroblasts and macrophages contain many lipid granules (black). Sudan IV stain. × 125.

FIG. 8. Photomicrograph of the anterior tibial muscle of a stock fed rabbit sacrificed 6 days after devascularization of the muscle. The cellular response is qualitatively and quantitatively the same as in the hyperlipemic rabbits, but the endomysial fibroblasts and macrophages do not contain lipid granules. Hematoxylin and eosin stain. × 125.



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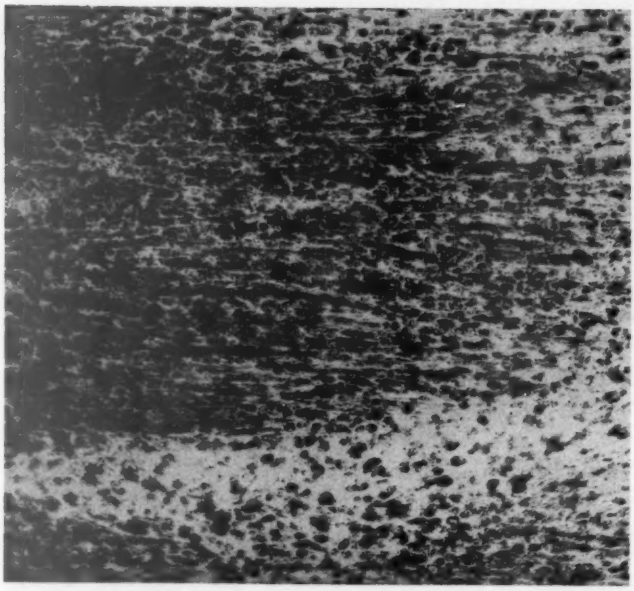

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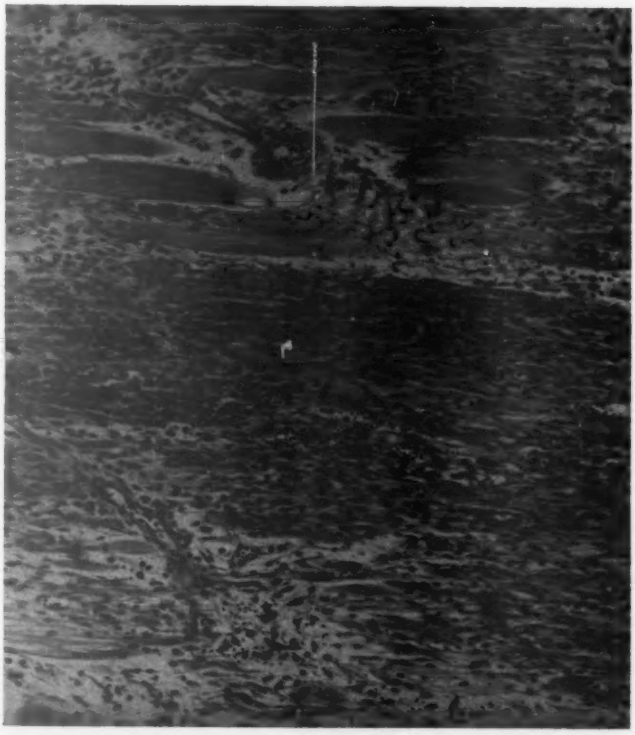

THE EFFECT OF HYPOPHYSEAL GROWTH HORMONE ON THE TIBIA OF THE DEVELOPING CHICK EMBRYO *

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This report is part of a series of investigations of the effects of pituitary growth hormone, administered during the last half of the embryogenic period, on the developing chick embryo. Initially it was shown that this hormone produces an increase in body weight and an elevation in blood sugar of the newly hatched chick, an effect similar to that observed in human fetal gigantism. These responses were more marked in females than in males.[1] Subsequent studies have demonstrated that the gain in weight may take place during the last 1 or 2 days of embryonal development.[2] It has been observed also that growth hormone increases the synthesis of pentosenucleic acid as evidenced by a greater quantity per embryo as well as a greater concentration by weight in both wet and dry-defatted tissue.[2]

It is our principal purpose in this report to correlate certain biochemical data with the rates of growth and cell division. Since the measurement of tibial bone growth has been used for assaying pituitary growth hormone, this appeared to be a favorable tissue for such a correlation. Such correlative data may be expected to contribute to an understanding not only of the rôle of growth hormone in normal bone development, but also of abnormal bone development and, in a larger sense, of the effects of this hormone on cell growth and division in general. These possibilities are indicated in the reports of Moon and coworkers[3] of an increased incidence of neoplasms in animals receiving hypophyseal growth hormone. The chick embryo affords a source of cells undergoing relatively frequent divisions, and the susceptibilities of these cells to hormonal stimulus may be compared with corresponding adult cells.

MATERIAL AND METHOD

Growth hormone† was injected chorioallantoically into New Hampshire eggs in three injections. The conditions of incubation and periods

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of injection were the same as previously reported.[1] Control eggs were divided into an uninjected group and one receiving alkaline saline solution (pH 8). Experimental eggs were divided into groups receiving total dosages of 5, 10, 20, and 40 μg. of growth hormone. Approximately 210 control and 540 experimental eggs were used. However, for simplicity of presentation, only saline injected controls and experimental eggs receiving 20 μg. of hormone are presented in most of the tables. At the appropriate periods, as indicated in the various tables, embryos were removed and tibias dissected and stored in a deep-freeze for subsequent chemical analysis and measurements. Tibias utilized for histologic examination were fixed immediately in 10 per cent formalin.

Three types of data were compiled: (1) bone measurements, (2) measurements of cell concentration and determination of mitotic counts, and (3) chemical analysis.

Tibial length was measured in a series of microscopic sections cut through approximately the mid-sagittal line at a thickness of 6 μ and stained with hematoxylin and eosin. Measurements were made with a microscope utilizing a calibrated mechanical stage. Bone width and thickness of epiphyseal disk were measured on fresh specimens by means of a calibrated micrometer ocular, utilizing a dissecting microscope.

Determinations of mitotic counts and cell concentrations were carried out on the sections used for measuring tibial length. The total number of mitotic figures per section, including periosteal cells as well as those in the growth zone, was recorded; in general, counts were made on three such sections for each bone and an average taken. The determination of cell concentration was based on the average number of cartilage cells in the growth zone per unit field; the latter was an arbitrarily squared off field on a glass disk inserted into the ocular of a microscope. Ten such fields were counted in each section and averaged; a larger average number of cells per unit field may be considered as indicating a smaller individual cell volume.

For chemical determinations, soft tissues were removed as thoroughly as possible from the bones and the tibias weighed on a precision balance and kept in a deep-freeze for analysis. The bone weights are recorded separately in Table I. The tibias were digested in 10 N sulfuric acid and made up to volume with water for analysis of total nitrogen, phosphorus (orthophosphate), and calcium. Total nitrogen was determined by micro-Kjeldahl procedure, phosphorus by a modified Fiske-Subbarow method,[4] and calcium by the method of Clark and Collip[5] as with blood serum.

TABLE I

Effect of Hypophyseal Growth Hormone on Tibial Size and Weight*

Incubation day	Bone weight		Tibial length		Tibial width					
					Proximal head		Center		Distal head	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
	mg.†	mg.†	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
14	21.3±1.6 (6)	27.1±1.6 (6)	9.5±0.6 (6)	10.3±0.4 (12)	1.7±0.6 (6)	1.8±0.3 (6)	0.95±0.3 (6)	0.97±0.8 (6)	1.3±0.4 (6)	1.6±0.2 (6)
15	39.8±1.4 (6)	39.8±1.3 (6)			2.3±0.3 (6)	2.3±0.2 (6)	1.2±0.4 (6)	1.4±0.2 (6)	2.0±0.4 (6)	2.1±0.2 (6)
16	48.2±2.8 (6)	54.6±1.3 (6)	11.7±2.6 (6)	12.2±1.5 (12)	2.4±0.3 (6)	2.8±0.3 (6)	1.3±0.3 (6)	1.5±0.3 (6)	1.9±0.2 (6)	2.1±0.2 (6)
17	74.7±3.4 (6)	93.6±3.4 (6)			2.9±0.3 (6)	2.8±0.4 (6)	1.3±0.2 (6)	1.2±0.2 (6)	2.5±0.3 (6)	2.6±0.1 (6)
18	119.9±4.2 (18)	133.1±3.5 (18)	14.6±1.4 (6)	15.3±0.7 (12)	3.0±0.5 (12)	3.4±0.5 (12)	1.4±0.3 (12)	1.4±0.4 (12)	2.7±0.4 (12)	3.0±0.5 (12)
19	217.3±6.4 (14)	234.0±5.7 (16)	20.0±1.2 (6)	20.3±1.0 (12)	4.4±0.9 (12)	4.7±0.9 (8)	2.0±0.3 (12)	1.9±0.5 (8)	4.4±0.9 (12)	4.9±0.7 (8)

* 20 μg. dose level.

† Weight of both tibias.

Figures in parentheses indicate numbers of animals.

Results

The data in Table I show that administration of growth hormone results in a distinct increase in tibial weight, which persists through the period of hatching; the experimental group shows a greater weight than controls on all incubation days observed except the 15th. This hormone probably also produces an increase in bone length, although the results are not as striking as for weight, and there is little difference between controls and experimental animals at the time of hatching. Similarly for measurements of width, injected animals, on the average, have wider bones than controls, but the differences are generally small. In Figure 1 is shown a comparison of the lower extremities below the knee joint of 18-day control (left) and experimental animals (right); while there is relatively little difference in length, the differences in girth are quite striking. However, a large part of this difference is apparently due to an increase in the volume of soft tissues in experimental animals; close observation of the two groups in Figure 2 (controls, left; experimental, right) shows slightly longer and broader bones in the injected group, but the differences are small as compared with Figure 1.

The results of measurements of epiphyseal disks are similar (Table II). Large doses of hormone appear to produce only a slight increase

Table II

Tibial Epiphyseal Measurement and Cell Concentration of Growth Zone of Newborn Chicks Injected with Growth Hormone

Group	Width of epiphyseal disk		Average number of cells per unit field of growth zone
	Male	Female	
	mm.	mm.	
Sham operated	5.09±.11* (19)	4.98±.07 (12)	12.8±1.3 (6)
Saline controls	4.89±.13 (9)	5.09±.14 (8)	13.9±1.7 (6)
5 μg. growth hormone	5.06±.14 (16)	5.03±.10 (15)	15.2±1.4 (6)
10 μg. growth hormone	4.99±.08 (18)	5.27±.05 (14)	16.6±1.8 (6)
20 μg. growth hormone	5.17±.06 (13)	5.14±.10 (15)	15.0±1.1 (9)
40 μg. growth hormone	5.22±.05 (26)	5.15±.13 (12)	16.6±1.3 (6)

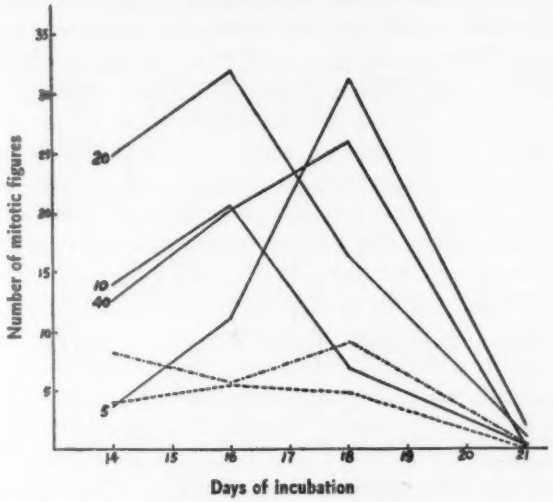
* Standard error.

Figures in parentheses indicate numbers of animals.

in the thickness of the epiphyseal disk by the time of hatching, and females appear to be slightly more sensitive than males.

Effect of Hypophyseal Growth Hormone on Mitotic Activity and Cell Concentration in the Growth Zone

The curves in Text-figure 1 illustrate the marked effect of growth hormone on mitotic activity. Mitotic figures were distributed almost equally between the growth zones and the periosteal tissues. Maximum responses were observed between the 16th and 18th days, but neither the degree of mitotic response nor the time of maximum mitotic activity was related to dosage, although it is possible that relationships between



Text-fig. 1. Effect of growth hormone on mitotic division in the tibia of the developing chick embryo. The results in the sham operated embryos are shown graphically by the broken line at the bottom, and in saline control embryos, by the curve in dots and dashes. Figures at the beginning of each solid curve represent dose in micrograms.

the various doses of hormone and period of maximum response would have been closer if counts had been carried out on the 17th incubation day. It is noteworthy that at the time of hatching both control and experimental groups showed low mitotic activity of similar degree. Despite the latter occurrence, certain effects resulting from mitotic activity remained: the last column in Table II, denoting cell concentration in the growth zones, shows greater average concentrations in experimental groups than in controls.

Effect of Hypophyseal Growth Hormone on Calcium, Phosphorus, and Nitrogen Concentrations of the Tibia

The results of chemical determinations are shown in Table III. It is noteworthy that the pattern of nitrogen concentration followed gen-

erally the curves of mitotic activity as well as those of nucleic acid content of the total embryo, as previously reported,[2] in that there was a gradual increase in nitrogen per unit weight of bone until a maximum was reached on the 18th incubation day; following this there was a fall in concentration. The controls showed generally higher levels than embryos receiving growth hormone. On the other hand, the calcium

TABLE III

Effect of Hypophyseal Growth Hormone on Calcium, Phosphorus, and Nitrogen Concentrations of the Tibia*

Incubation day	Nitrogen		Calcium		Phosphorus	
	Control	Experimental	Control	Experimental	Control	Experimental
	mg. %	mg. %	mg. %	mg. %	mg. %	mg. %
14	1.25±.07 (5)	1.36±.07 (6)			1.62±.04 (6)	1.49±.07 (6)
15	1.49±.03 (6)	1.43±.05 (5)	0.72±.07 (4)	0.24±.01 (6)	1.59±.07 (6)	1.86±.06 (6)
16	1.62±.05 (6)	1.54±.08 (6)	1.86±.18 (5)	1.58±.08 (5)	1.32±.12 (5)	1.29±.09 (6)
17	1.89±.04 (6)	1.76±.10 (6)	1.78±.13 (6)	2.02±.08 (6)	1.28±.02 (5)	1.36±.07 (6)
18	2.76±.37 (12)	2.55±.28 (12)	2.17±.16 (12)	2.56±.14 (12)	1.35±.05 (12)	1.48±.03 (12)
21	2.37±.06 (44)	2.35±.10 (28)	3.93±.17 (43)	3.88±.11 (33)	1.78±.05 (51)	1.76±.03 (27)

* 20 μg. dose level.
Figures in parentheses indicate numbers of animals.

concentration of the tibia increased progressively through the period of hatching. The quantity of calcium per unit weight of bone was distinctly lower in experimental groups on the 15th and 16th incubation days, and higher than controls on the 17th and 18th incubation days, while at the time of hatching there was little difference between the two groups. The data on phosphorus showed a progressive fall in concentration between the 14th and 17th incubation days, followed by a subsequent rise which was particularly sharp between the 18th day and hatching time. Embryos receiving growth hormone showed fluctuations in phosphorus concentrations of the tibia up to the 18th incubation day. These values were sometimes higher and sometimes lower than control levels, but except for early periods, were generally higher when calcium was higher. Again, there was a sharp increase between the 18th and 21st incubation days, and at the time of hatching there was little difference in phosphorus concentration between the tibias of control and experimental groups.

Effect of Growth Hormone on Histologic Structure of the Tibia

By the 14th incubation day, when observations were begun, the tibia is formed and consists of a rounded mass of cartilage at each head, surrounded by a thin capsule of perichondrium; the zone nearest the ends of the bone represents articular cartilage, while the deeper layers comprise the growth and proliferating zones. No columnar arrangement of cells representing an epiphyseal disk is noted until some time between the 19th and 21st incubation days, and unlike the long bones of mammals epiphyseal centers of ossification are not observed. Deeper within the shaft from both ends of the bone there are irregular areas of degenerating and calcifying cartilage in process of conversion to bony trabeculae. Marrow spaces with developing hemopoietic cells are present by the 14th incubation day, when a distinct periosteum has formed and from this bony trabeculae are developing.

Two distinct differences were noted between the tibias of control and experimental animals between the 14th and 18th incubation days. Mitotic activity of the cartilaginous growth and proliferating zones was more marked (*cf.* Figs. 3 and 4) in embryos receiving growth hormone, probably accounting for the differences in bone length. Growth hormone also produced a marked proliferation of periosteal cells and increased periosteal bone formation (*cf.* Figs. 5 and 6); the latter probably accounted for the differences in bone width, and both of these for the increased bone weight. Although the cartilage cells of the growth zone were somewhat larger in embryos receiving growth hormone between the 16th and 18th incubation days, by the 21st day, when all groups showed a low rate of mitotic activity, these cells were smaller and more compactly arranged in experimental animals. In all other respects the bones of the two groups were similar. No developmental defects of the bones were observed.

Discussion

The most striking effects of growth hormone on chick embryonic bone (tibia) are an increase in weight and a marked increase in mitotic activity of both the cartilaginous growth zone and periosteum. The latter results in an increased concentration of cartilage cells within the growth zone. The differences in bone width and length between control and injected groups are least striking, and the increase in thickness of the epiphyseal disk is intermediate in deviation. The increase in concentrations of calcium and phosphorus after the 17th day in embryos receiving growth hormone suggests an increase in density of these tibias, and this may account in part for the increase in weight.

Greenbaum and Young[6] have reported an increase in size of all

organs, but particularly muscle, following the administration of pituitary growth hormone, an effect which Russell[7] attributed to the regulation of the conversion of amino acids to protein by this hormone. A comparison of Figures 1 and 2 appears to substantiate the observations of Greenbaum and Young of a disproportionate increase of soft tissues. On the other hand, the diminution in nitrogen concentration in the tibias of injected embryos would indicate a depression of protein synthesis in bone, while such synthesis may be increased by growth hormone in other organs and soft tissues. Our previous observation that growth hormone increases the pentosenucleic acid concentration of the whole embryo would tend to substantiate such a conclusion, since Brachet[8] and also Caspersson[9] have observed that when there is a state of active protein synthesis there is a marked accumulation of pentosenucleic acid in all cells, and Di Stefano *et al.*[10] have noted that the administration of growth hormone enhances the accumulation of this nucleic acid in liver cells. Therefore the effect of growth hormone on the chick embryo is probably a differential one with regard to protein synthesis, increasing its rate in organs and soft tissues while depressing it in bone.

These observations and conclusions are particularly important with regard to the biochemical changes associated with mitotic activity. Growth hormone administration, as demonstrated here, leads to an increased mitotic activity in bone, and we have observed a similar effect on other organs. The use of the term growth in designating this hormone may be misleading, since this term designates an increase in mass, volume, area, or length of cells, tissues, or organisms which can take place without cell division at all. The growth effect in the sense of enlargement of cells or organs may also follow upon the primary effect of increasing cell division. As one of us has pointed out,[11] mitotic division may precede or follow the advent of various nutrient elements to the cell. The trigger mechanism which initiates mitotic activity is not known. Hughes[12] stated that there may be one key-point at which metabolism of the cell may be altered regularly by the application of a special stimulus in the direction leading inevitably to mitosis. On the other hand, Berrill[13] does not regard cell division as the result of a single stimulus, but "the climax of a complex process of growth and duplication of a multitude of cell components, while the inhibition of the process in vivo is clearly not due to lack of nutritive substances or to any deficiency on the part of the cell." Needham[14] is of the opinion that the initiating factor is probably no more than the proper conjunction of nutrient materials and the appropriate capacities for making use of them. It is of interest, therefore, that increased

mitosis can occur in localities where protein synthesis probably is inhibited. However, another consideration must be taken into account: Blumenthal and Loeb[15] have pointed out that in the adrenal gland, mitotic activity occurs under conditions in which the lipids of the cell are depleted, thus suggesting that certain nutrient components may act as inhibitory agents, and growth hormone may therefore exert its mitosis-stimulating effect through such a mechanism. Studies now in progress indicate that total fat increases sharply in the embryo between the 17th incubation day and the time of hatching, the period during which mitotic activity also falls off sharply; the total lipid level is lower at all stages in embryos receiving growth hormone than in controls.

Finally, the site of action of growth hormone on embryonic bone is of interest. The observations of Freud, Levie, and Kroon,[16] as well as Ray, Evans, and Becks,[17] indicate that the effect of growth hormone is not confined to the epiphyseal disk, but influences the whole process of endochondral ossification and coincident connective tissue metaplasia. Ray, Evans, and Becks stated that the effect of pituitary growth hormone on normal periosteal ossification is less marked. This is contrary to the effect observed in the chick embryo, in which the effect on the periosteal tissues is at least as great as that on the cartilage of the growth zone.

Summary

Growth hormone (Armour) administered chorioallantoically to chick embryos in three divided doses during the last half of embryogenesis results in a striking increase in weight and in mitotic activity of the tibia. Mitotic figures occur with approximately equal frequency in the growth zone and in periosteum. Increased cell division of the growth zone results, at hatching, in an increased concentration of cartilage cells in both the proximal and distal heads.

A less striking increase in bone width, bone length, and thickness of the epiphyseal disk was observed.

An increase in calcium and phosphorus concentrations of the tibia also was observed after the 17th incubation day, suggesting an increased density of bone, which would account, at least in part, for the increase in bone weight.

Despite the increase in mitotic activity following hormone administration, nitrogen concentration is lower in the tibias of experimental embryos than in controls.

These observations are discussed in relation to the mechanisms by which this hypophyseal hormone may increase growth and initiate mitotic activity.

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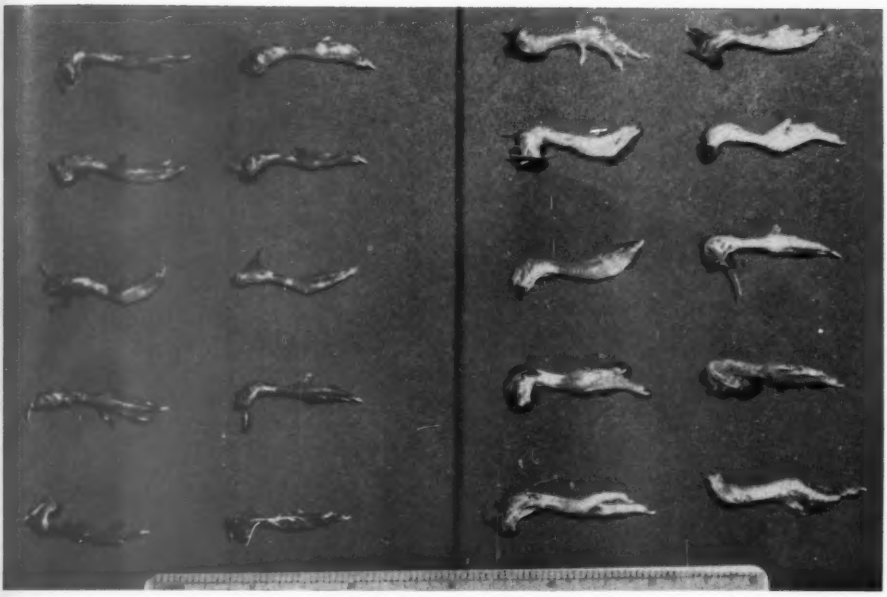
LEGENDS FOR FIGURES

FIG. 1. A comparison of the size of the lower extremities below the knee joint of 18-day control (left) and growth hormone injected (right) chick embryos. Of note is the marked enlargement of the extremities of the experimental animals.

FIG. 2. A comparison of the size of the tibias of 18-day control (left) and growth hormone injected (right) chick embryos. There is only slight, if any, enlargement of the tibias of experimental animals.



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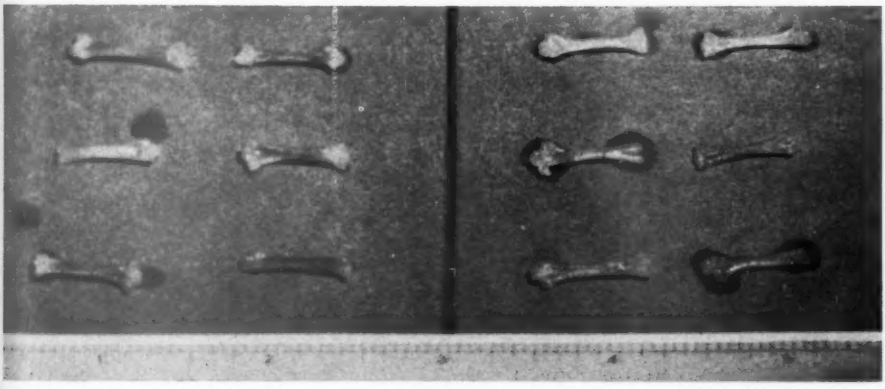

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FIG. 3. Cartilaginous growth zone of a tibia of an 18-day control chick embryo, showing small, regular nuclei and absence of mitotic activity. × 400.

FIG. 4. Cartilaginous growth zone of a tibia of an 18-day growth hormone injected chick embryo. Enlarged nuclei and mitotic figures are indicated by arrows. × 400.

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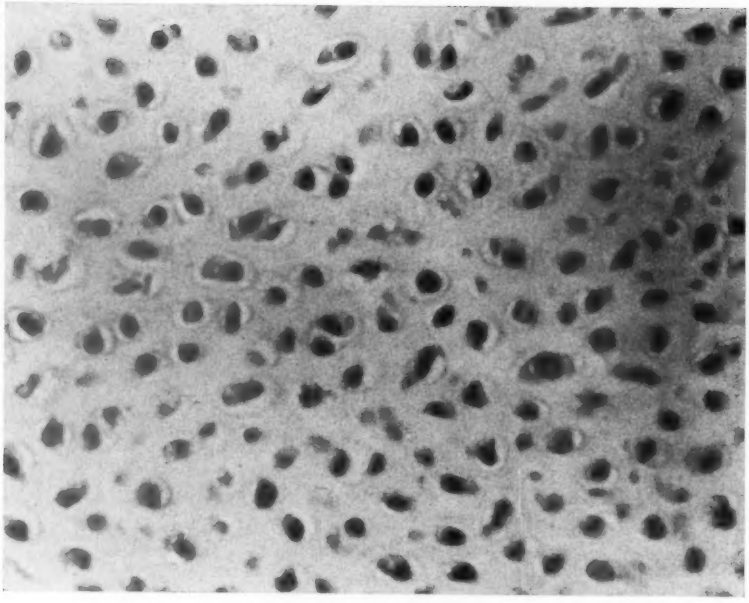

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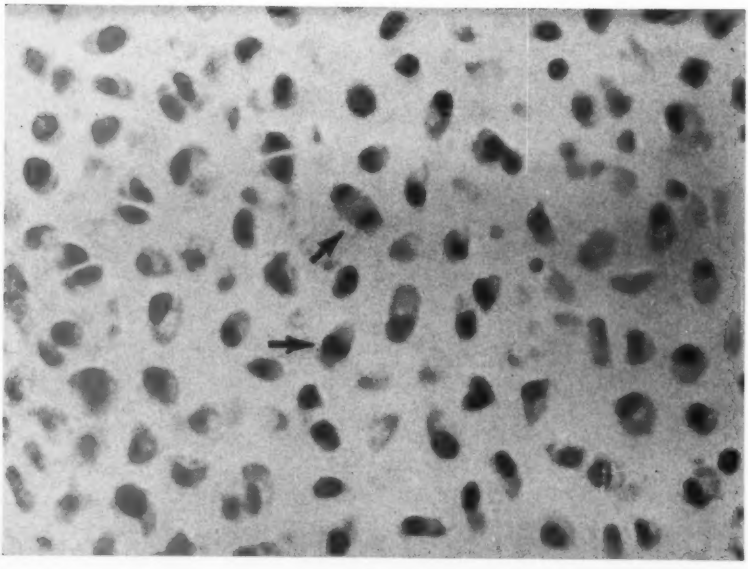

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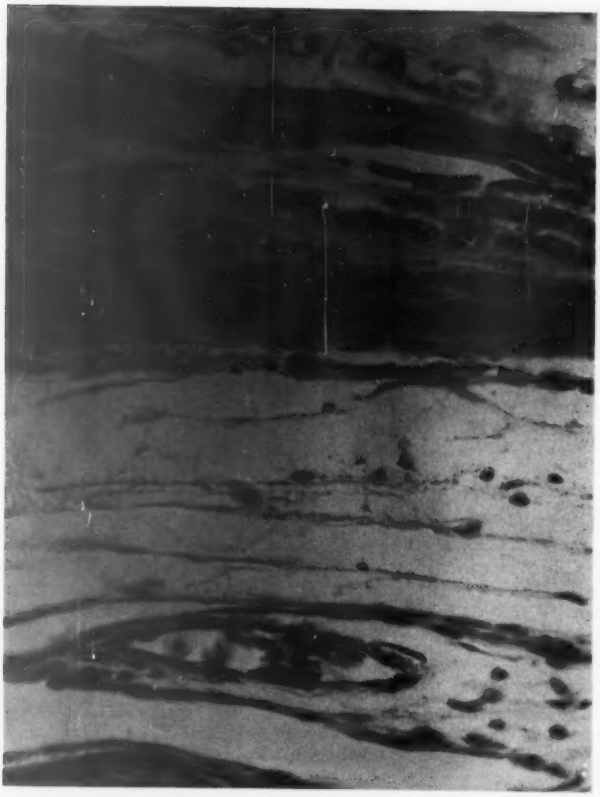
FIG. 5. Periosteal area and adjacent fat of a tibia of an 18-day control chick embryo, showing small compressed periosteal cells and a thin zone of periosteal bone formation. × 400.

FIG. 6. Periosteal area of a tibia of an 18-day growth hormone injected chick embryo. There is a thick proliferating periosteal zone, only a portion of which is shown in the photomicrograph. Arrow indicates a mitotic figure. × 400.



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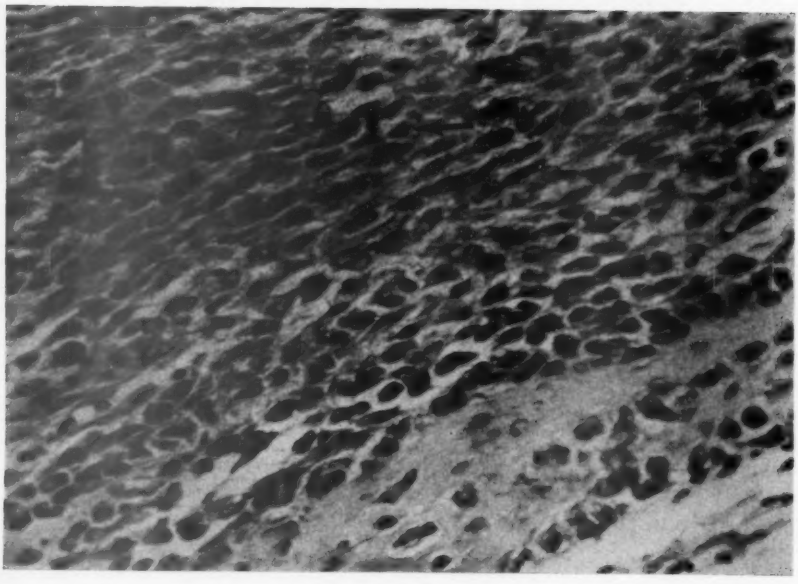

6

GIANT HYPERTROPHY OF THE JEJUNAL CIRCULAR RUGAE ASSOCIATED WITH RECURRENT INTUSSUSCEPTION *

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The intestinal lesion described herein is rare and, so far as we can determine, hitherto unreported. It is characterized by a tremendous hypertrophy of the jejunal circular rugae and by a segmental involvement. The principal clinical feature in the case to be presented was recurrent episodes of small intestinal intussusception. The question arises as to whether the lesion may be the cause or the result of the recurrent intussusception. Furthermore, is the lesion similar morphologically to giant hypertrophy of the gastric rugae (Menetrier's disease)? An attempt is made to answer these questions.

REPORT OF CASE

An Indian girl, 19 years old, registered at the Kiowa Indian Hospital in May, 1953, and gave a history of severe abdominal pain associated with persistent vomiting and absence of bowel movements for 3 days. At laparotomy the surgeon found an intussusception involving the jejunum about 2 feet from the ligament of Treitz. The intussusception was reduced manually. The intussusceptum and intussuscipiens, measuring together about 3 feet in length, were resected and submitted to the National Institutes of Health for pathologic study.

The patient had had two previous proved episodes of intussusception, each necessitating surgical reduction. The first episode occurred in November, 1948, and the second in October, 1952. The acute symptoms were similar to those described during the third episode. The first, third, and perhaps the second episode of intussusception involved the same segment of upper jejunum. The second intussusception occurred in the small intestine, but in view of the patient's condition exploration to determine the exact location was not attempted. During the first and third operations the surgeon could find no cause for the intussusception, though the mucosal folds were described as being edematous and redundant.

The patient first noted symptoms referable to the gastro-intestinal tract late in 1947. The clinical picture was of abdominal pain with or without associated nausea and vomiting. These symptoms were of transitory nature. Though usually mild, they were of sufficient severity to necessitate hospitalization in January, 1948, in March, 1948, and in May, 1949. During these hospitalizations, the symptoms subsided with supportive treatment. The more severe illnesses may have been due to jejunal intussusception with subsequent spontaneous reduction. However, at that time the patient did not follow through with the advised investigation of the gastro-intestinal tract by roentgenologic procedures.

A gastro-intestinal roentgenologic examination was performed 2 months after the surgical removal of the intussuscepted portion of the jejunum. No abnormalities

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The term jejunal circular rugae denotes the circular or transverse folds of mucosa in the jejunum and is synonymous with the terms valves of Kerkring, folds of Kerkring, valvulae conniventes, and plicae circulares.

were found in any segment of the gastro-intestinal tract. The patient was seen 5 months after the operation and stated that she has been free of symptoms.

The patient first entered the hospital in 1946 for a tonsillectomy. At that admission a history of having congenital syphilis was obtained. Though stigmata of syphilis other than a positive serologic test were absent, penicillin therapy was administered.

The family history was not pertinent.

Morphologic Observations

The surgical specimen (S-36387), fixed in 10 per cent formalin, was a segment of jejunum measuring 75 cm. in length, 10.5 cm. in circumference at one resected end, and 7.5 cm. in circumference at the other resected end. The segment of jejunum included both the intussusceptum and the intussuscipiens. Both ends of the specimen exhibited no gross abnormalities. The mucosal surface along a distance of 50 cm. presented a segmentally distributed lesion of unusual appearance (Fig. 1). In six discrete areas the circular mucosal folds were thickened irregularly to a width over 1 cm. and to a height varying from 1 to 4.5 cm. The largest area of involvement measured 13 by 12 cm. The tremendous hypertrophy of the circular rugae gave rise to a few, coarse, secondary folds which spread out superficially in various directions (Fig. 2). These giant folds resembled papillomatous structures with narrow or broad stalks and cauliflower-like tops. Thick, tenacious mucus often covered the surface of the folds but was not considered excessive. The hypertrophied folds, on section, presented a redundant, non-thickened mucosa which was freely movable upon an edematous submucous layer of varied thickness. In the involved segments the intestine was greatly dilated but did not possess ulcers, infarcts, or hemorrhage. The attached mesentery contained multiple lymph nodes varying in size from 0.3 to 1.5 cm. The mesenteric vessels appeared normal.

Microscopically, the lesions presented marked enlargement of the jejunal circular rugae (circular folds or valves of Kerkring). These hypertrophied rugae were composed of redundant mucosa, muscularis mucosae, and submucosa (Fig. 3). The mucosa was intact and measured in thickness from 0.4 to 1 mm. Except for superficial foci of autolysis, the epithelium showed no alterations in histologic structure (Fig. 4). The lamina propria contained eosinophils, plasma cells, and lymphocytes in numbers considered to be within normal limits. At a few sites the lamina propria presented solitary follicles of lymphatic tissue. Hyperplasia of these follicles and a surrounding infiltration of lymphocytes occasionally gave rise to larger nodules of lymphatic tissue which involved the superficial portion of the submucosa, the muscularis mucosae, and the lamina propria (Fig. 5). The muscularis

mucosae, except where obscured by lymphatic tissue, was distinct and of average thickness. The submucosa made up the bulk of the hypertrophied folds. It consisted of richly vascular, edematous connective tissue (Figs. 4 and 6). The latter occasionally contained a faintly eosinophilic, homogeneous substance situated between the connective tissue fibrils. Special stains for the demonstration of mucopolysaccharides (mucicarmine; Rinehart modification of the Hale procedure; periodic acid Schiff; and pH 4 thionine) failed to reveal reactive material in the interfibrillar, intercellular zones. Both frozen and paraffin sections were used. Because of the marked edema, the submucosa appeared sparsely cellular. The cell types included only fibrocytes, histiocytes, and mast cells.

In the submucosa at the base of the hypertrophied folds there was focal lymphangiectasia (Fig. 7). Dilatation of lymphatic vessels occurred also in the hypertrophied folds proper but was less conspicuous there (Figs. 4 and 6). The dilated lymphatic channels consisted of a thin endothelial lining and of a lumen free of fibrin and other stainable material except for occasional lymphocytes. The connective tissue surrounding the lymphatic vessels was edematous and did not contain fibrin, inflammatory cells, or excessive fibrous tissue. The small arteries and arterioles appeared normal. Thrombi and hemorrhages were absent. The muscularis showed no anatomical alterations except for segmental separation of the inner muscle coat by edema (Fig. 7). The serosa was thickened focally by vascular fibrous tissue which contained a few lymphocytes and macrophages. Elsewhere it appeared normal. In the grossly non-involved portions of the jejunum the only pathologic changes were slight submucosal edema and lymphangiectasia. The mesenteric lymph nodes presented hyperplastic follicles and slight hyperplasia of the sinus reticulum. The mesenteric blood vessels were engorged, but no thrombi could be found. The mesenteric lymphatic vessels often were dilated to a varying extent and were free of parasitic involvement.

Discussion

The case described is of an unusual intestinal lesion characterized by marked enlargement of the jejunal circular folds. Hence, the term giant hypertrophy of the jejunal circular rugae. The hypertrophy was based principally or entirely upon redundancy of the mucosa and edema in the submucous layer. The mucosa was not thickened.

In gross appearance the lesion resembled large papillary adenomas. However, upon microscopic examination of the area it was found that the mucosa did not show hyperplasia, metaplasia, or neoplasia. The hypertrophied folds usually were devoid of inflammatory cellular in-

filtrate. Except for occasional sites of lymphocytic infiltration, the submucosa possessed only fibrocytes, histiocytes, and mast cells. These cells, as well as the collagen and elastic fibers of the submucous layer, were separated by a precipitate free of fibrin, inflammatory cells, and stainable mucopolysaccharide material. It is apparent, then, that the submucosa was thickened by a transudate. The precipitate was colorless or occasionally faintly eosinophilic, suggesting a low protein content.

Where present, the foci of lymphatic hyperplasia contributed minimally to the hypertrophy of the circular folds. The lymphatic tissue was localized and involved, at most, the lamina propria and superficial portion of the edematous submucosa. O'Sullivan and Child[1] reported two cases of ileocecal intussusception caused by localized lymphoid hyperplasia in the ileum. However, the degree of lymphoid hyperplasia, as illustrated, was much more marked than in the present case.

Of particular interest is the relation of the hypertrophied jejunal folds to the recurrent episodes of intussusception. That a relation existed is evident from the fact that two, and perhaps three, of the episodes involved the same segment of jejunum. This was determined in each instance by surgical intervention. At neither of two operations could the surgeon find a cause for intussusception, though the mucosa was described as redundant. At the third operation the intussusceptum, intussuscipiens, and portions of non-involved jejunum were removed. The only anatomical lesion found was that which has been described. Is this lesion, then, the cause or the result of the intussusception? Decisive evidence upon which to base the answer to this question is not available.

It seems possible that much of the pathologic change could be dependent upon the effects of lymphatic obstruction. This would explain the lymphangiectasia present in the submucous layer of the hypertrophied folds as well as the submucosal transudate. The causes of lymphatic obstruction may be considered under two principal headings: inflammatory and non-inflammatory. The present case lacked features which would suggest an inflammatory process, such as cellulitis and lymphangitis. Furthermore, there was no parasitic permeation of the lymphatic channels of the intestinal wall or mesentery. In the non-inflammatory group, lymphatic obstruction may result by the following processes: (1) invasion of lymphatic vessels by a malignant neoplasm; (2) pressure upon the main lymphatic vessels by a neoplasm; (3) neoplasm originating from lymphatic vessels; (4) mechanical torsion or bending of the main lymphatic vessels; and (5)

malformation of the lymphatic vessels. Neoplasia was absent in the present case. Thus, the first three items are not pertinent.

The fourth process, mechanical torsion or bending of lymphatic vessels, is speculative. Conceivably, this could originate from torsion or stretching of the mesentery involved in the intussusception. However, it is doubtful that the resultant lymphatic obstruction would be sufficiently diffuse or complete enough to cause the markedly enlarged jejunal circular folds. Such an appearance has not been observed to occur in other cases of recurrent intussusception. If mesenteric torsion or stretching was a significant causal factor, one would expect to see a concomitant vascular impairment with resulting hemorrhage and infarction. This was not found. The fourth process would leave unexplained the segmental distribution of the jejunal lesion and the cause of the intussusception. The fifth process, that of malformation of lymphatic vessels or, for that matter, of the jejunal wall itself, is a possibility which cannot be proved or discarded. Until additional cases with lesions of this type are studied, a solution to the problem of etiology is wanting.

The anatomical configuration of the giant hypertrophied folds in the jejunum is suggestive of a particular method of growth from less enlarged mucosal folds. Sunderland and Binkley[2] stated that the mere projection of any rounded mass from the intestinal wall seems to act as a foreign body, and the activity which it elicits may be forceful. Conceivably, peristaltic propulsion in the intestine could create a mechanical drag on a small sessile lesion. If the lesion was a compact mass of tissue, such as a tumor not adherent to the muscular layer, one would expect it to be dragged out on a pedicle. However, in the present case the mucosa was redundant and mobile over an edematous submucous layer. A mechanical drag upon such a lesion may lead to a papillomatous structure of giant hypertrophied folds composed of the mucosa, muscularis mucosae, and submucosa. Further mechanical drag upon the enlarged folds could induce the intussusception. This, in turn, through additional mechanical trauma and possible lymphatic obstruction, may aggravate the existing pathologic process. If this be the mode of development of the giant hypertrophied jejunal folds, the etiology of the less enlarged folds still remains obscure.

So far as is known, the peculiar lesion of the jejunum reported here has not been described before. However, it does resemble morphologically a rare gastric condition first described by Menetrier[3] in 1888 as *polyadenoma en nappe*. Other terms applied to the lesion include giant hypertrophic gastritis and giant hypertrophy of the gastric rugae.

The cause of this condition is unknown. Maimon *et al.*[4] described six cases and discussed the clinical and pathologic features of the entity. Their description of the lesion and their illustrations clearly depict the variations in the pathologic features. In all of their cases, redundancy of the mucosa was a constant finding and gave rise to large mucosal folds which resembled cerebral convolutions or papillomatous structures. Inconstant findings included: hyperplasia or hypertrophy of the mucosa; alteration of the glandular structure with replacement of the pepsin and acid-forming cells by intestinal goblet cells; cystic glands in the mucosa; thickening, increased vascularization, lymphangiectasia, and edema of the submucosa; fibroplasia of the mucosa and submucosa; and nests of normal mucosal epithelial cells herniated through the muscularis mucosae. Each of these changes was extremely variable in regard to its frequency of occurrence in particular specimens.

It is apparent that giant hypertrophy of the gastric rugae (Menetrier's disease) has several features in common with giant hypertrophy of the jejunal circular rugae. Both conditions are characterized by redundancy of the mucosa with the formation of giant folds. Both present edematous changes in the submucous layer of the folds, though this finding appeared to be more striking in the jejunal lesion. Hypertrophy of the mucosa, alterations in the glandular cells, and fibroplasia of the mucosa and submucosa often are prominent features of Menetrier's disease but were absent in the present case. Though both pathologic processes may possess the same salient morphologic features, their relationship is obscure. The hypertrophied gastric rugae in Menetrier's disease could not be based causally upon intussusception, for the latter usually has not been demonstrated to occur. In only two of the six cases reported by Maimon *et al.*[4] was vomiting a prominent feature. This apparently resulted from acute pyloric obstruction due to prolapse of the redundant mucosa through the pylorus. If Menetrier's disease is related in pathogenesis to the present case, it may be argued that intussusception played no rôle in the origin of the jejunal lesion.

Summary

A peculiar pathologic entity involving the jejunum is described. It is characterized by prominent hypertrophy of the jejunal circular rugae (valves of Kerkring) and by a segmental distribution. The enlarged rugae are composed of redundant, non-thickened mucosa, muscularis mucosae, and an edematous submucous layer. Inflammation and neoplasia are absent.

This condition, termed giant hypertrophy of the jejunal circular

rugae, was associated in the case reported with three episodes of small intestinal intussusception. It seems likely that the lesion elicited the intussusception, though the effects of the latter may have aggravated the existing pathologic process.

The pathogenesis of the entity is unknown. It is possible, however, that the giant mucosal folds developed from the mechanical dragging effect of intestinal peristalsis upon less enlarged sessile folds.

The present case possesses several morphologic features in common with the rare condition called giant hypertrophy of the gastric rugae (Menetrier's disease). However, the exact relation between the two is obscure.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Upper portion of jejunum showing a segmental distribution of giant hypertrophic circular rugae. There is dilatation of the intestine in the involved areas. Formalin-fixed material.

FIG. 2. Enlargement of an area from the left side of Figure 1. The hypertrophied circular rugae assume a papillomatous structure with narrow or broad stalks and cauliflower-like tops. Uninvolved portions of the intestine may be seen in the lower half of the photograph.

FIG. 3. Entire histologic section of a group of giant hypertrophied jejunal folds. Both ends of the section reveal relatively normal jejunal wall. Of note are the redundant, non-thickened mucosa and the edematous submucous layer. Hematoxylin and eosin stain. × 2.5.

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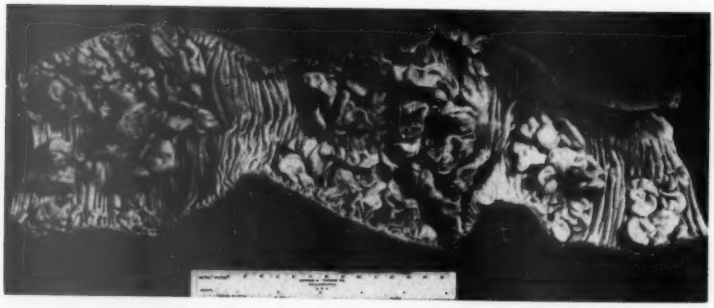

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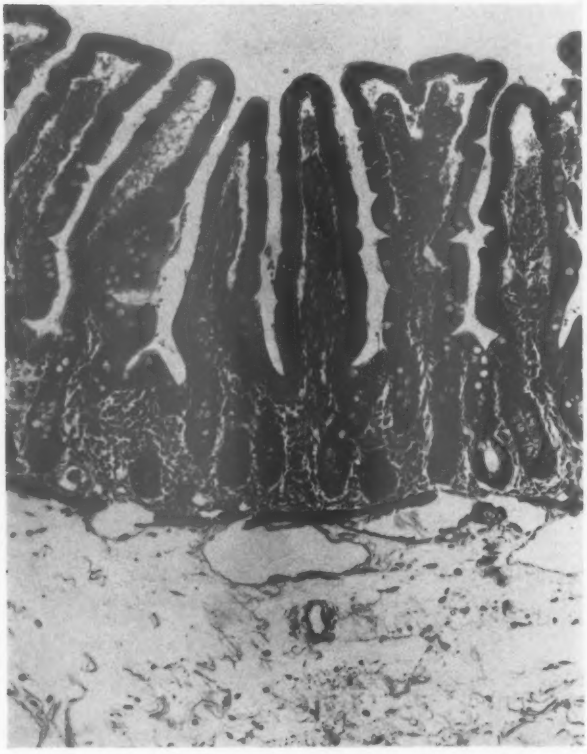
FIG. 4. Mucosa and underlying portion of the submucous layer from a hypertrophied jejunal circular fold. There are no alterations in the mucosa. The separation of the lamina propria from the villous epithelium is an artifact. The muscularis mucosae is present and not thickened. Slightly dilated lymphatic channels are present in the edematous submucous layer. Hematoxylin and eosin stain. × 115.

FIG. 5. Mucosa and portion of the submucous layer from a hypertrophied jejunal circular fold showing lymphatic dilatation and a lymphocytic hyperplasia with infiltration of the superficial submucosa, the muscularis mucosae, and the lamina propria. Hematoxylin and eosin stain. × 30.

FIG. 6. Mucosa and a portion of the submucous layer from a hypertrophied jejunal circular fold showing a non-altered mucosa, an intact muscularis mucosae, and a vascular, edematous submucosa free of inflammatory cells, fibrin, and fibrosis. This represents the usual picture of the hypertrophied folds. Rinehart modification of the Hale stain. × 30.

FIG. 7. Base of a hypertrophied jejunal circular fold showing greatly dilated lymphatic vessels, venous congestion, edema of the submucous layer with absence of inflammatory cells and of fibrosis, edematous separation of the inner muscular coat, and slight thickening of the serosa. Rinehart modification of the Hale stain. × 30.



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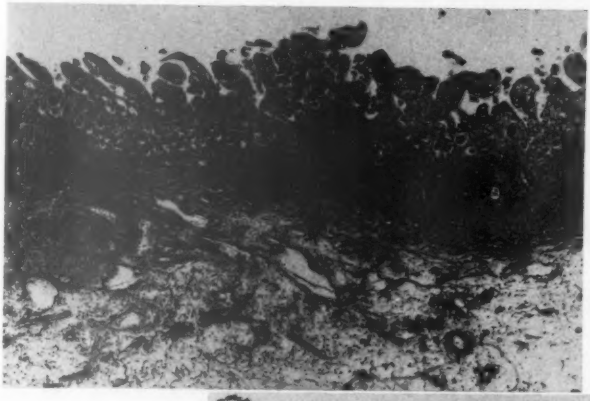

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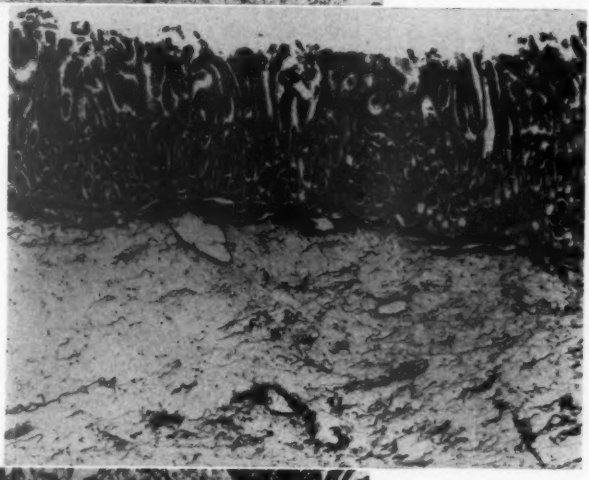

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EXPERIMENTAL PRODUCTION OF PIGMENTED VILLONODULAR SYNOVITIS IN DOGS *

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In 1865 Simon[1] reported the first case of xanthoma of the knee joint, and since then many terms have been applied to this unusual reaction of synovial tissues. These terms include xanthoma, villous arthritis, hemorrhagic villous synovitis, benign and malignant giant cell tumor, xanthogranuloma, myeloplaxoma, and pigmented villonodular synovitis. The last, a descriptive term coined by Jaffe *et al.*,[2] has gained general acceptance. Minear,[3] summarizing the reported cases, found that males were more frequently affected, the age group of greatest incidence was 20 to 40 years, the location was principally in the lower extremity with most cases showing involvement of one knee, the duration of symptoms usually covered years, and a history of trauma was obtained in a significant number of cases. Many authors have concluded that trauma is a cause of this lesion, though the mechanism is not understood.

The lesion is usually described as a focal or diffuse process. Grossly, there are hypertrophied villi and nodules of small or large size, and these are often deeply pigmented reddish brown. The villi and nodules are most numerous in the recessed portions of the joint but may involve most of the synovial surface. The villi are often described as moss-like. The nodules are usually firm and deeply colored. Microscopically, the villi may be discrete or show fusion along their margins, producing slit-like synovium-lined spaces. The cores of the villi are very vascular and may show scattered round cells and hemosiderin-laden macrophages. The villi may show hyaline change and scarring in the older lesions. The synovial lining also contains hemosiderin. The nodules often show scarring, the slit-like spaces described, focal areas of degeneration and hemorrhage, focal collections of giant cells and macrophages, and hemosiderin deposits. There may also be areas of cellular fibrous proliferation.

For a number of years one of us (J. M. Y.) has believed that trauma is a very significant factor in the etiology of villonodular synovitis. This trauma often is not marked and may not bring the patient to the doctor. The injuries are of the type which produce instability of the

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joints and set the stage for repeated minor or major recurrences which lead to repeated hemarthrosis and keep the lesion active. In our experience, a history of significant injury is almost always present. Villonodular synovitis may be present alone or in combination with other pathologic joint changes such as torn cartilages, loose bodies, or osteochondritis dissecans. Our patients have usually been heavy-set or large individuals who were prone to bear with their disability until the lesion had produced marked interference with joint function. This accounts partly for the long course which allows full development of the lesion. With these considerations in mind, the following investigations were performed to evaluate the rôle of repeated hemorrhage into joints as a cause of pigmented villonodular synovitis.

Material and Methods

Twenty full-grown mongrel dogs weighing 20 to 35 lb. were used in this experiment. In 10, preliminary operative procedures were performed. These operations were for the purpose of creating small and medium-sized defects in the articular cartilages of the tibia, femur, and patella. Since there was no difference in the responses of the joints in the operated and non-operated dogs, no separation of these two groups will be made.

The experiment covered approximately 1 year. During the first 6 months biweekly injections of autogenous blood were made into the knee joint and Achilles tendon. For the next 3 months weekly injections were performed, and during the last 3 months injections were given three times each week. The blood used in each animal came from its own jugular vein and was not citrated or defibrinated. The blood was drawn and injected immediately by use of a 20 cc. syringe and an 18 gauge needle. In the beginning 4 cc. of blood were introduced into the knee joint and 2 cc. into the Achilles tendon. Later, as the joint capacity increased, more blood was injected into the knee joint so that near the end of the experiment 10 to 15 cc. was the usual amount.

It became apparent that the injected blood was rapidly removed from the joints, and the animals used the injected member within 2 or 3 days following the injection. Six of the dogs died of intercurrent diseases before 6 months had elapsed. Examination of their joints disclosed only moderate villous hyperplasia. It was decided to give the injections at weekly intervals and later even more often.

The animals would not use the injected extremity until most of the blood had been removed. In the first few months this interval was 2 or 3 days, but later the animals stopped using the injected extremities altogether. They made no effort to use the extremity even to maintain

balance and kept the knee in moderate flexion. The range of joint movement was frequently tested and found to be normal or to show minimal reduction, though a full range of movement often produced pain.

Results

Joint Injections

Gross Appearance. Examination of the joints of the 6 animals which died within the first 6 months disclosed moderate villous hyperplasia of the synovium, usually along the margins of the menisci, in the intercondylar notch, around the margin of the patella, and in the posterior recesses of the joint. There was slight fibrous thickening of the joint capsule and slight brownish discoloration of the synovium, most prominent where the villous hyperplasia had occurred. The degree of villous hyperplasia was most marked in the joints which had been recently injected. No nodule formation was noted in these joints. The villi were fine and thread-like and showed fusion in a few places.

The remaining 14 dogs were sacrificed at periods ranging from 8 to 13 months from the beginning of the experiment. As the number of injections increased there occurred more marked fibrosis of the joint capsule and slower removal of the injected blood. When the joints were opened the amount of fluid varied from 4 to 15 cc. The fluid was always thick, viscid, and xanthochromic, and the degree of coloration depended on the time interval from the last injection to the date of sacrifice. The synovium in every joint examined disclosed brownish discoloration to a greater or lesser extent. The joints receiving the largest number of injections had the deeper shades of pigmentation. In 4 of the animals sacrificed from 2 to 8 days after the last injection, blood clots were floating free in the fluid. In most of these joints clots had also become attached to the synovial surface and disclosed varying degrees of organization; some were sessile and others pedunculated. In 2 animals, organized and hyalinized nodules floated free in the fluid.

The degree of villous hyperplasia varied with the time interval from the last injection. In the joints recently (2 to 8 days) injected the villi were plump and turgid and measured as much as 2 cm. in length. They were most prominent and numerous in the recesses and folds of the joint, as was mentioned. In joints where the last injection had been 10 to 20 days before, the villi were thread-like and slim, but of comparable length. Where the time interval from last injection to sacrifice was more than 1 month, the number and the length of the villi decreased moderately. In the joints which received the injections three times weekly, many of the villi were bulbous and more deeply pigmented, as was the joint capsule. Many of the villi were firm and fibrous. Fusion of villi was common, producing a mesh-like pattern.

Often the surface presented a pebbly appearance. The parts of the joint capsule in contact with the moving surfaces were smooth and the least pigmented. In the quadriceps pouch attached clots and fused villi were common, and strands of synovium and adhesions formed bridges from one surface to another.

Nodules formed in two ways. The first was by attachment and organization of blood clots. The second was by fusion of adjacent villi. The nodules formed by attached and organized thrombi were most numerous in the quadriceps pouch, while those formed by fusion of villi were most numerous along the peripatellar and meniscus margins and in the intercondylar notch and popliteal regions. The nodules usually were firm and light or dark brown. Some had dark reddish zones where the blood clot had not yet resolved, producing a streaked appearance.

The joint capsule was thickened in every instance, measuring as much as 0.5 cm. in places. It, too, showed brownish discoloration on the cut surface. The lymph nodes in the popliteal region were enlarged and their cut surface disclosed a light brown color. The lesions which have been described were produced in all of the animals surviving more than 6 months.

Microscopic Appearance. The villi of the joints of animals dying in less than 6 months disclosed a loose cellular fibrous core which was very vascular. The synovium was hyperplastic and varied from 3 to 8 cells in thickness. Hemosiderin was present in small amounts in the synovium and in macrophages in the stalks of the villi. Scattered plasma cells and lymphocytes were present, usually in the perivascular regions. There was a minimal increase in the fibrous tissue of the joint capsule.

The joints of the animals sacrificed 8 months or longer after the beginning of the experiment presented varied microscopic changes. In many areas villi were similar to those described and were hyperplastic and newly formed. Older villi presented more scarring, ranging from slight cellular fibrosis of the core to complete hyalinization. Fusion of adjacent villi was common, leaving irregular synovium-lined spaces. The fusion of villi and the organization of attached blood clots produced a thick pannus of tissue over parts of the joint capsule. Deep in this pannus were scattered large and small synovium-lined spaces. The organization of the thrombi, depending on the stage of the process, produced very cellular to hyaline changes. Often the fibroblastic response was marked with considerable variation in size of nuclei and cells. The attached thrombi were frequently pedunculated and organized into a nodule which gradually underwent complete hyalinization. There were scattered multinucleated giant cells in these and the other

nodules. Collections of lipid-laden macrophages were fairly frequent in these nodules and in the organizing pannus of the capsule. Synovium covered over the sedimented blood and clots to exclude these from the joint. Fibrin and red blood cells were often present on the surface of the villi. There were no significant changes in the articular cartilages other than those created by the original operation.

Hemosiderin was present in the synovial and deeper layers. The amount present was dependent on the number of injections and the time interval from the last injection to date of sacrifice. Where injections had been frequent and recent, hemosiderin deposits were marked. The iron staining material was in the synovial cells and in the deeper tissues. Many hemosiderin-laden macrophages were in the sub-synovial tissues and joint capsule, and many had carried hemosiderin to adjacent lymph nodes. Where the time interval from last injection to date of sacrifice had been greater than 1 month, hemosiderin had diminished greatly in amount, but had not disappeared.

ACHILLES TENDON INJECTIONS

Gross Appearance. Injections of blood into the Achilles tendon resulted in local extravasation and fibroblastic response. Grossly, there was scarring about the bundles of the tendon and a greater or lesser degree of brownish discoloration. No true nodules or tumor-like aggregates of tissue were formed. The zone of fibrosis formed a dense ring about the tendon and extended into the adjacent tissues.

Microscopic Appearance. Varying infiltration with hemosiderin-laden macrophages was present, depending on the frequency and date of injections. A few collections of cholesterol crystals with a surrounding foreign body reaction were encountered. No synovial clefts were formed. A few collections of lipid-laden macrophages were present in the fibrous tissue where breakdown of hemoglobin and tissue had been recent.

Failure to produce the tendon sheath equivalent is probably because the Achilles tendon has only a vestigial sheath. Attempts will be made to develop the lesion in other locations.

Discussion

Early investigations of synovial reactions to blood were carried out by Key[4] in 1929. He found that a single injection of citrated blood into the joints of rabbits produced an inflammatory response and hypertrophy of the villi. The inflammatory response was marked at first and was accompanied by prominent vascular engorgement. The reaction had almost completely subsided in 5 days. The inflammatory response was with polymorphonuclear cells at first, and these were

gradually replaced by round cells and macrophages. The synovial proliferation was most prominent around the margins of attachment of the cartilages and in the pouches and folds of the synovial sac. When Key used multiple injections (seven in 24 days), similar but more pronounced changes occurred. He noted light brown pigmentation of the synovium in some areas, increase in capacity of the joint, small blood clots attached to the synovial surface, and fringes of hyperplastic synovium and villi. The joints in these animals were examined at intervals up to 12 days, and it is significant that though reversion toward normal had occurred, the synovium still showed increased numbers of villi, thickening of the capsule, and hemosiderin-laden macrophages 12 days after injection.

Injections of blood, cholesterol, and ferric chloride into rabbit joints were performed by Jaffe *et al.*[2] who concluded that these materials did not produce pigmented villonodular synovitis, though details of the experiments were not given. Injections of materials other than those mentioned would seem to have little meaning in this condition, since the products in the reaction are lipids, blood, and tissue breakdown compounds.

It seems clear that the presence of blood in joints is essential for the production of pigmented villonodular synovitis. The initial injury or precipitating factors produce intra-articular hemorrhage which is followed by proliferation of villi. These villi remain large as long as blood or excess fluid is to be removed. If the patient ambulates at this point he could easily damage and crush some of the villi by joint motion. This would lead to more hemorrhage and more pronounced synovial hyperplasia, thus setting in motion a self-perpetuating process.

There are other indications that trauma is the important factor in the production of pigmented villonodular synovitis. The lesion occurs most often in the knee or ankle, important weight-bearing joints of the lower extremity. In the event of any instability of these joints they are thus more likely to be injured, especially in heavy-set or large individuals. Once the condition has developed, periods of rest result in improvement with decrease or disappearance of effusion, pain, and limitation of motion. Ambulation may bring a recurrence, especially if injury again happens.

Bennett,[5] in clinical specimens, noted red blood cells and fibrin attached to villi and postulated that they result from damage to villi, and that organization of these leads to intervillous adhesions and increasing scar tissue and hemosiderin deposition. The presence in joints of blood which has not been citrated or defibrinated leads to formation of clots which can be organized. Our animals showed fairly numerous pedunculated organized blood clots similar in gross and histologic appearance

to some of the nodules in clinical cases of villonodular synovitis. In two animals some of these had become detached to form loose bodies within the joint.

Other conditions giving pronounced villous hyperplasia could conceivably set the stage for development of pigmented villonodular synovitis, so an initial injury need not always happen. The patient then causes damage to the villi, again producing hemorrhage, and a continuous self-perpetuating cycle is started. With periods of rest, the joint improves, but because of continued use a recurrence is likely and the process is activated again. The end result is some degree of pigmented villonodular synovitis which may be present alone or complicate other injuries such as cartilage tears or osteochondritis dissecans.

The question may be raised as to why patients with hemophilia do not show this lesion. Perhaps this is explained by four reasons. First, the blood of hemophiliacs is not as likely to clot within the joint, thus allowing more rapid and complete removal. Second, these patients usually seek medical treatment early and are more likely to receive prolonged therapy because of their disease. Third, these patients have most of their joint difficulties in childhood and at younger ages when the joints of the lower extremities do not bear much weight. Fourth, inspection of the synovium of joints in hemophiliac patients is not likely to be carried out while the process is active, and with rest much of the fully developed picture disappears. Joints of patients with hemophilia do not disclose at necropsy the picture of the synovial reaction seen shortly after repeated hemorrhages. Likewise, changes in the articular cartilage and subchondral bone in hemophiliacs produce great joint deformity and complicate the picture even more. With damage to bone and articular cartilage there is resulting loss of motion and activity which favors the regression of any villonodular component of synovitis.

Nevertheless, Key,[6] in 1932, described the histologic changes in the knee of a hemophiliac patient which are usually found in pigmented villonodular synovitis, indicating that the active phase of the synovial reaction is very similar to that of pigmented villonodular synovitis.

De Santo and Wilson[7] found recurrence of the lesion in 7 of 41 surgically treated cases. This would indicate as more likely a recurrence of conditions which produced the lesion in the first place rather than any intrinsic neoformative potentiality in the synovial tissue. They further emphasized that no case of pigmented villonodular synovitis has been reported to undergo malignant change. The opinions expressed in the literature are that this lesion is not a true neoplasm and that it has no malignant potentialities. This experiment indicates that prompt removal of bloody effusion from joints is desirable. The

patient with an unstable joint must realize his limitations and take precautions to protect the joint. Once the lesion has fully developed, surgery seems indicated to remove the thick pannus. Roentgen therapy probably has its most pronounced effect during the proliferative phase of the process.

Conclusion

By experimental methods lesions of joints similar to those in pigmented villonodular synovitis have been produced. Repeated hemorrhages into the joint are essential for the development of this lesion, and trauma is often the initiating factor.

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LEGENDS FOR FIGURES

Fig. 1. Dog knee joints for comparison. Injections given for 10 months. The quadriceps pouch is prominent.

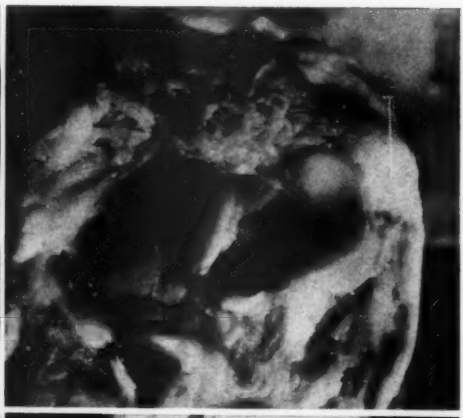
Fig. 2. Tibial surface of joint injected for 6 months. Elongated villi may be seen along the anterior tibial margin. No nodule formation is present.

Fig. 3. Tibial surface of a joint revealing pannus and nodule formation in the upper left corner. Many villi have become flattened and fused to form the pannus.

Fig. 4. Quadriceps pouch filled with pedunculated and sessile nodules resulting from organization of blood clots. Villi are present around the patella and joint margins.

Fig. 5. Sagittal section through a knee joint to show thick pannus in quadriceps pouch. There is a pad-like accumulation of tissue in the joint space beneath the patellar region. With joint motion this tissue could easily be damaged between articulating surfaces.

Fig. 6. Same joints as in Figure 5. Quadriceps pouch is now on the left side. The joint has been spread open to reveal the pronounced villous hyperplasia and early nodule formation.



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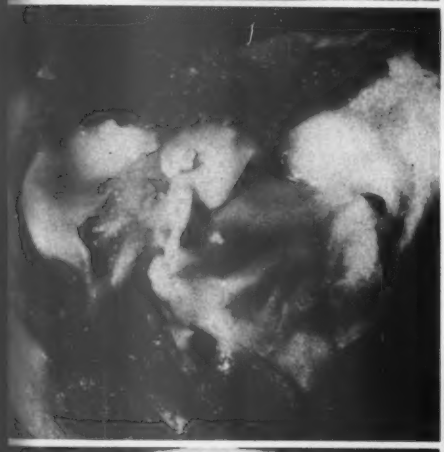

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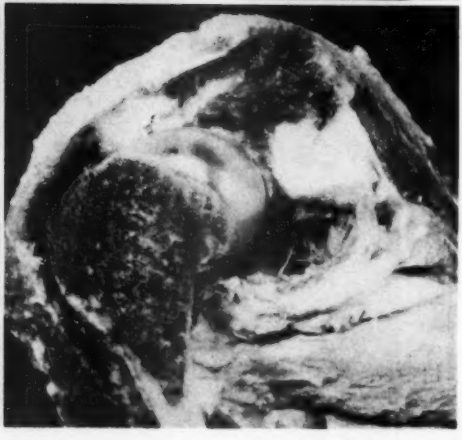

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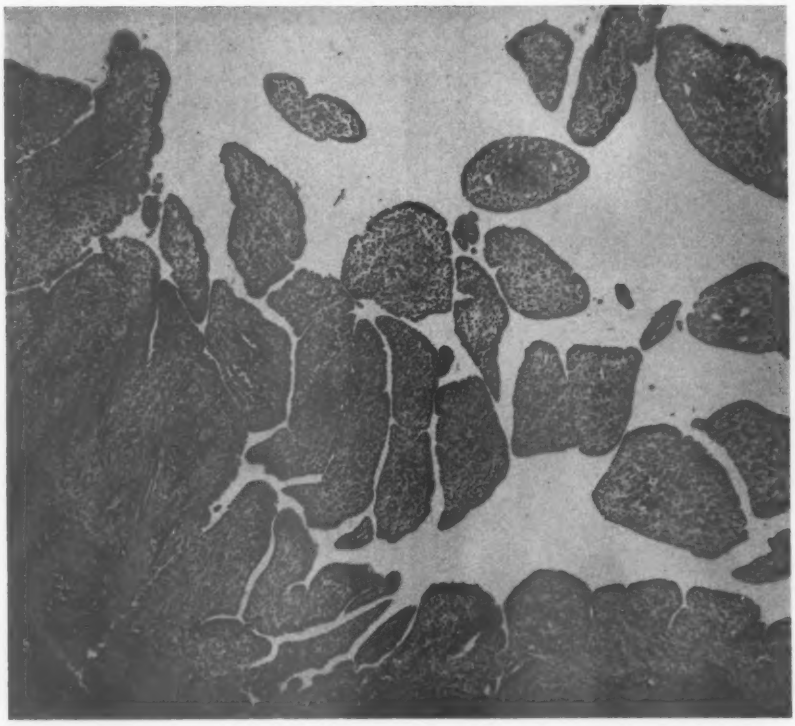
FIG. 7. Villous hypertrophy and hyperplasia. Animal sacrificed after 8 months of injections. Beginning fusion of villi may be noted. Hematoxylin and eosin stain. × 60.

FIG. 8. Proliferative phase of villous hyperplasia, showing fusion and numerous slit-like spaces lined by synovium. Some hyaline change has occurred in the stroma, and a few collections of chronic inflammatory cells are present. Much hemosiderin is present in the synovium. Hematoxylin and eosin stain. × 100.

FIG. 9. Joint capsule showing dense cellular and hyaline fibrosis and synovium-lined clefts in the wall; some are very deep near the outer part of the capsule (bottom center). Hemosiderin deposition is marked in the capsule and synovium. Hematoxylin and eosin stain. × 60.



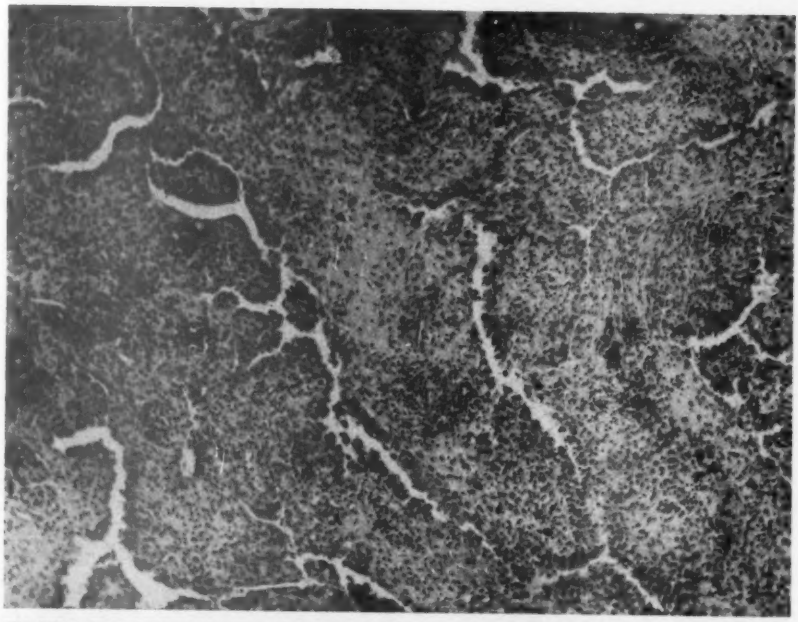


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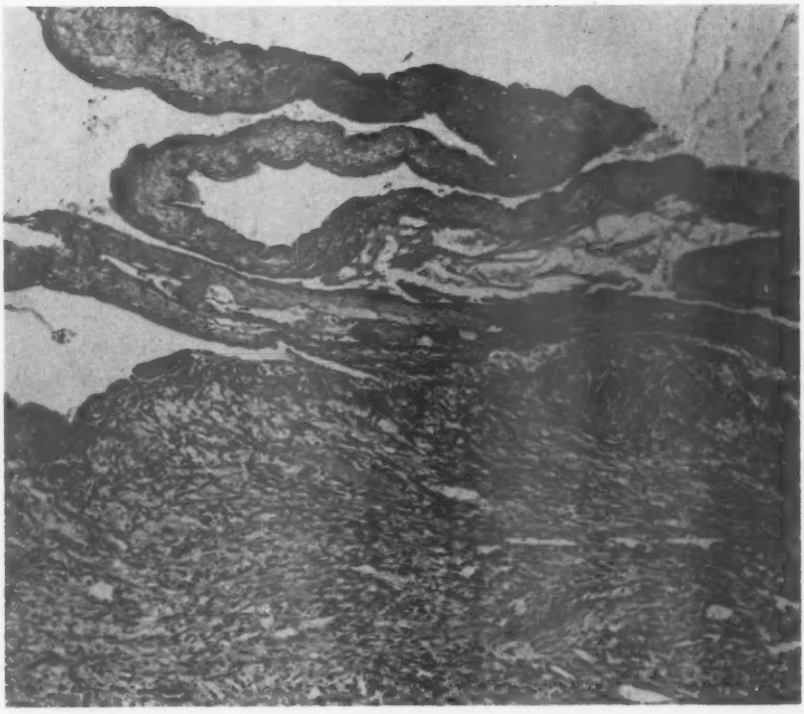
FIG. 10. Organization of sedimented blood and fibrinous material. Of note is the desmoplastic fibrous reaction. Many lipid-laden and hemosiderin-laden macrophages are scattered through this tissue. Hematoxylin and eosin stain. × 100.

FIG. 11. Low-power magnification of a nodule showing scattered giant cells and cellular and hyaline fibrosis. Hematoxylin and eosin stain. × 30.

FIG. 12. High-power magnification of a nodule to show giant cells, cellular fibrosis, and numerous macrophages which contain hemosiderin. Hematoxylin and eosin stain. × 100.



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LUNG MITES

Pulmonary Acariasis as an Enzootic Disease Caused by Pneumonyssus Simicola in Imported Monkeys *

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Reports of lesions caused by mites in the lungs of monkeys have appeared sporadically over the years, but there has been little investigation of the pathogenesis of the disease, or of the life history of the mites. The condition, because of its almost constant occurrence in monkeys necropsied (more than 400†) by us in the past few years, merits attention from the point of view of chemotherapeutic control. The lesions must have been seen innumerable times in institutes where rhesus monkeys are used experimentally. It is difficult to conceive that our experiences have been unique.

The number of monkeys imported annually into the United States must be huge, possibly over 30,000 a year, and most seem to be imported from India. Probably more are used for experiments in which necropsies are not done, or the thorax not opened; the lesions (and the causal mite) may not have been recognized as such, but more likely they may have been confused with other diseases, or the lesion noted and cursorily considered to be insignificant. In papers dealing with experiments on monkeys there is virtually no casual reference to this disease, even incidental to the titled experiment. The parasitic lesions cannot be considered, however, as minor and intercurrent, especially if monkeys are used in any studies associated with pulmonary changes, such as tuberculosis. In any work on mycobacterial infection, confusion might arise, by non-recognition of the mite lesion for what it is, regarding the extent and spread of the tuberculous process.

Regulations exist concerning importation of animals into various countries, to prevent introduction of previously non-existent diseases. Examination of monkeys at the port of entry, however, can be only cursory, and cannot be designed practically to reveal whether monkeys are harboring non-indigenous internal parasites, some of which might even be disease vectors.

This paper is a recounting of facts, some of which have been described previously, which will help focus attention on a problem of

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† This number includes 280 monkeys upon which a preliminary survey of the incidence was made in our laboratories but not published, by Maj. J. MacNamee, Paul P. Yevich, and A. Dorothy Bergner (1950–51).

concern to monkey importers, experimentalists, and zoos. Our contribution is, however, based on a large series of necropsies of monkeys (over 400) as compared with other records on the disease in a few animals. Ewing[1] gave a complete description of the genus Pneumonyssus in 1929. It is apparent that various species had been identified in the lungs of monkeys as early as 1901. An article by Helwig[2] was about the first to deal adequately with the pathology, although Murray[3] had reported the condition previously in monkeys in the London Zoo. Gay and Branch[4] and Dormanns[5] made contributions on a few cases and Hamerton,[6] working in the London Zoo, also described the condition briefly. Monlux and Turk[7] referred to the occurrence of *Pneumonyssus caninum* in the facial sinuses of a dog in Texas. From these various papers, and Chandler,[8] almost all known references dealing with lung mites in various animals and birds can be obtained. Helwig referred to various reports on identification of mites in the urine, sputum, and tissues of human beings (see also Carter, Wedd, and D'Abrera[9]). Chandler mentioned that another genus of mites (Halarachne) infests the lungs of seals and sea lions, and yet another (Entonyssus) of snakes, but we are unaware that any lesions were found or studied. Infestations of the air sacs, air passages, and even air cavities in the bones of chickens, turkeys, and game birds by yet another genus of acari (*Cytodites nudus*) are common, and well known to avian pathologists (Biester and Schwarte[10]).

Personal Studies on Pulmonary Acariasis in Monkeys

In the past few years over 400 monkeys have been subjected to necropsy examination in our laboratories (including those by MacNamee, Yevich, and Bergner, 1950–51). Nearly all of these animals were *Macaca mulatta,* and most of them had died, or had been killed, after use in toxicologic experimentation; but many were natural deaths from various causes. The necropsies of over 120 monkeys, 1952–53, included a deliberate search for the lesions of pulmonary mites, apart from determination of general pathologic findings. In the total group lesions were found in all but 20 animals. More significant, however, was the discovery of some degree of the disease in all monkeys examined during 1952–53 when the lesions were specifically sought. It is possible that a meticulous search, aided by the use of the dissection microscope on thin slices of fixed lung and extensive histologic examination, would almost always reveal lesions and perhaps the mites as well.

CLINICAL FINDINGS

By casual inspection of living monkeys, clinical signs of pulmonary disease may not be detectable, or may be only those of moderate at-

tacks of sneezing and coughing. It has hence been impossible to decide clinically whether any given living monkey will show no, a few, or disseminated pulmonary lesions at necropsy. There are, consequently, intrinsic difficulties which might vitiate experimental work with mites on imported monkeys, which vary in size, weight, age, and condition, and on purchase are, in fact, animals with an unknown clinical history.

It seems inescapable that mites, by their wanderings in the lungs, must irritate the bronchial passages and interfere with normal health, but apparently this is not severe enough to produce florid clinical pulmonary signs, except on rare occasions. Many monkeys, in which multiple pulmonary lesions were found at necropsy, appeared to be in good bodily condition, and would have been considered by most experienced workers as excellent examples of Macaca, fit for any experiment.

All monkeys received in our colony are subjected to tuberculin testing, and in the earlier work by MacNamee, Yevich, and Bergner (1950–51), various organs and the mite lesions were examined for the presence of acid-fast mycobacteria, but with negative results. Spontaneous tuberculosis has not been observed in our monkey colony during the past few years, and once the mite lesions are recognized for what they are, there can be no confusion with those of any form of simian tuberculosis.

From the point of view of experimental biologists using monkeys, it would be desirable to know whether living animals are affected with lung mites before starting an experiment. Inasmuch as clinical observations were valueless, roentgenologic examination was considered as a possible diagnostic aid. Full anteroposterior and lateral views of the thoracic cage were taken of 6 monkeys, the plates being exposed immediately after euthanasia by nembutal to avoid chest movement. The roentgenograms were later compared with the anatomical distribution of pulmonary lesions. In one case 18 distinct pulmonary foci were found at necropsy, of which 3 were present in the right upper lobe. However, detection of such lesions with certainty in the roentgenograms was literally impossible; with the exception of one minute area of opacity in the upper right lobe the remainder of the film appeared normal. Even roentgenograms of lungs dissected out of the thorax showed nothing in the way of punched-out disseminated opaque foci. Consequently, routine roentgenograms of the chests of monkeys in a normal colony, or by importers, would be of no diagnostic value.

Since the pulmonary lesions are parasitic in origin, a prominent participation of eosinophilic leukocytes in the histologic reaction was suggestive that this might be reflected in an eosinophilia of the pe-

ripheral blood. This is not evident. Complete routine hematologic examinations have been made, for other reasons, on very many monkeys passing through our hands. It is rare to find any marked degree of eosinophilia, particularly that which can indubitably be correlated with the presence of mite lesions. In one group of 25 monkeys, upon which complete hematologic studies were done, the average eosinophil count was 3.7 per cent in a total average white cell count of 12,000 per cmm.; only 2 monkeys showed a figure of more than 4 per cent eosinophils, these being 18 and 17 per cent respectively. In another 15 animals, the average eosinophil count was 0.73 per cent in an average total white cell count of 21,200 per cmm., the highest eosinophil count being 4 per cent. Eosinophilia is thus not a constant, or even a common, finding, as it should be if it were related to the presence of mites in the lungs. Even if present, other internal parasites, *e.g.*, the common helminthic infestations in the alimentary canal, might be partly or wholly responsible.

PATHOLOGIC DESCRIPTION

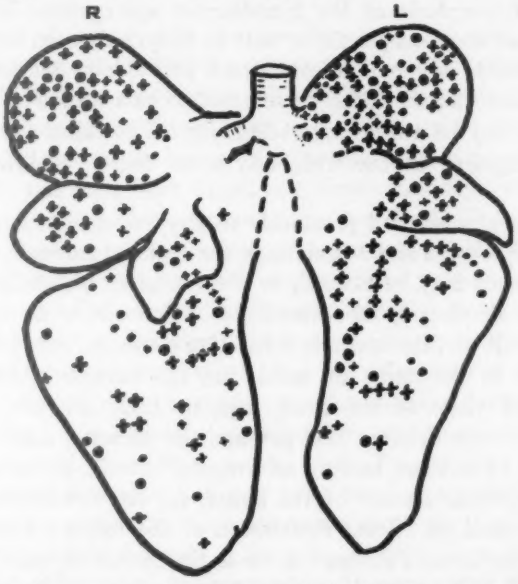
The following descriptions are based on the many monkeys which have now been examined, and which thus include many hundreds of individual lesions.

Macroscopic Findings (Figs. 1, 2, and 3)

The parasitic nidus of inflammation is discrete, and may seem to be almost always subpleural, but this is not truly so. If the lungs are fixed and then sliced, foci may be found in any part of any lobe. They may be just within macroscopic visibility as minute pale spots, midst normal pink, crepitant, lung tissue. Most range from this pin-point size up to lesions a few millimeters in diameter, which are yellowish in fresh tissue. Some, but not all, are elevated above the normal pleural surface, and may extend into deeper tissue for 1 to 2 mm. Superficially they may thus simulate some hematogenous mycobacterial lesions, but they are not firm like tubercles. Infrequently they are surrounded by a congested zone. Under the dissection microscope, foci appear as pale white, jelly-like masses, in the centers of which may be a minute slit or round opening. (In some the opening may be more irregularly enlarged, or it may even appear as a small cyst.) In the white tissue there is always an irregular marbling effect produced by the presence of a yellowish material and a black pigment. The latter may also show as a delicate, lace-like, ramifying network scattered throughout adjacent normal lung. Few lesions become approximated and confluent, and no clear example has been found where the morbid process had spread by contiguity to result in a lobar pneumonia; but

a few monkeys have shown more extensive lobular areas of consolidation with localized pleural adhesions. The regional hilar and bifurcational lymph nodes show no gross changes apart from pigmentation. There is no concomitant pathology in the other organs of the thorax and abdomen.

In some lungs only a few (2 or 3) foci are present, while in others there may be as many as 50, or more. The lesions were counted in the lungs of some monkeys, and a great variation in the numbers was found. Their distribution was also plotted, and the results are illustrated in diagrammatic form (Text-fig. 1). The causal mites apparent-



Text-fig. 1. Pulmonary acariasis, monkeys. Plotted distribution of lesions in lungs based on the examination of many monkeys; cross represents lesions visible on anterior surface and dot, those visible posteriorly; approximately 50 per cent are in the apical lobes.

ly migrate in a somewhat fortuitous fashion to any part of the lungs. Yet, in a total of 270 peripheral lesions, seen from the surfaces and mapped into a composite sketch, there appears to be some predilection for lodgment in the apical lobes; 127 were present on the right side, 143 on the left, and about 50 per cent of this total involved both apical lobes; 182 were visible on the anterior surfaces and 88 on the posterior.

Microscopic Findings

No matter where the pulmonary foci are situated, they have a classical structural pattern, which cannot be confused with any other focal, or disseminated, disease of man or lower animals. Most lesions

seem to have some connection with a bronchiole, and beyond a level in the tree at which cartilaginous plates start to disappear. In some sections, lesions seem to be embedded deep in lung parenchyma and not involving bronchioles—large, small, or terminal—but this probably is a matter of the level at which the lesions have been cut, as we have found in serial sections of selected cases. The fully developed process (Figs. 6 to 9) is one of restricted bronchiolitis and peribronchiolitis, which involves directly only adjacent alveoli to cause some degree of lobular collapse. The mite causes inflammation leading to a thickening of the bronchiolar wall; there is variable destruction of the lining epithelium, a reactive hyperplasia of the lymphocytic aggregations in the wall, and the small macroscopic slit or hole so frequently seen in the center of the advanced lesion is a constricted bronchiolar lumen. Only in some instances can the lesion be referred to as a walled-off cyst. The latter word may be a little misleading, for the so-called cyst is simply a dilated segment of the wall, *i.e.*, some degree of bronchiectasis (Fig. 9).

In monkeys unexposed previously to any experiment which results in pulmonary edema and hemorrhage, the regional parenchyma related to a mite focus may be normal, or show degrees of lobular collapse. When fully developed, the bronchiolar lesion shows an enormously thickened wall, and the lumen is reduced to a crevice, partly by detritus and mucus, in the midst of which lies the parasite; but in single sections only parts of the head, legs, or body are seen (Figs. 6, 7, and 8). Much detritus, and perhaps the parasite, may fall out in preparation of sections, leaving an irregular ectasia, or the latter may be a late intrinsic feature of the lesion, for the detritus and mucus may be coughed up. Great destruction of the lining epithelium may occur with the mucosa stripped down to the muscle layers. The thickening of the wall is largely due to inflammatory cellular exudate in which there is a mixture of cells including polymorphonuclear and eosinophilic leukocytes, lymphocytes, and macrophages; the number of eosinophils varies from lesion to lesion, and these cells may be predominant. Peribronchiolar lymphoid infiltrations may be pronounced, with formation of prominent follicles (Fig. 6). The distribution of the pre-existing muscle fibers of the bronchiolar wall may be irregular, but this perhaps is a matter of displacement of the fibers within the area of inflammation. Some degree of true proliferation of plain muscle fibers has been observed (Fig. 11).

Scattered throughout the lesion are macrophages crammed with golden brown to blackish pigment (and also refractile crystals) which form dense clumps in, near, and around the main focus (Figs. 10 and

12). This pigment can be scattered far and wide throughout the lung tissue, distant from any parasitic focus, but is then found mostly in phagocytes within perivascular and peribronchial lymphatics. Occasionally there are aggregations of pigment-bearing phagocytes lying free in otherwise normal air spaces, and some pigment may form extracellular masses. (Reference to the nature of the pigment is made later.) There is little or no tissue necrosis and no giant cell formation. Fibrosis is not a cardinal feature to the extent of forming dense collagenous bundles, but special stains reveal an anastomosing fibrillar reticulum ramifying through the bulk of the thickened inflamed bronchiolar wall.

In regions of focal lobular pneumonitis there is alveolar collapse, or the air spaces are consolidated and filled with exudate composed of all types of leukocytes, sometimes with eosinophils predominating, and pigment-bearing macrophages (Fig. 13). This focal pneumonitis without any mite may be the lesion found incidentally in monkey lungs.

Many lesions have been found in different monkeys which did not fit into the general pattern of the changes described. These were found in lungs in which there were relatively few macroscopic foci, and hence such lesions may represent early stages of the process. In these instances, the respiratory bronchioles and alveolar ducts, in very restricted segments of the bronchial tree, showed a striking degree of ectasia and far less inflammation (Fig. 9). The walls of these dilated structures showed slight to moderate infiltrations of lymphocytes and macrophages. These were often irregular, with some polarity as to the parasite and its location on the wall. The parasite was usually found in these instances, and may have been in a larval form. This inflammatory reaction involved adjacent alveoli, and the latter tended to be absorbed into the wall of the affected bronchiole. Lesions have been found which might be intermediate between these described and the advanced foci.

Many bronchioles proximate to a parasitic focus show little or no inflammation, but can present massive collections of pigment-laden phagocytes, mainly in the circumscribing lymphatics, which may indicate merely passage of pigment toward the hilum and regional lymph nodes. There is no constant striking vascular involvement around the foci, producing hyperemia, congestion, or edema in otherwise normal monkeys. Hemorrhage was not seen.

There is no metaplasia of the alveolar epithelium such as is seen in jaagsiekte, or in other chronic inflammations of the lungs of some domestic animals. The reaction is different from that produced in focal and lobar pneumonias caused by a variety of nematodes in lower

animals. In one monkey, small groups of unidentified nematode larvae were found in alveolar spaces not too distant from a mite lesion, and, as in some stages of helminthic invasion of ruminant lungs, there was no surrounding inflammatory reaction. There was no case with clear-cut extension of the process terminating in a parasitic lobar pneumonia, although instances of confluent bronchopneumonia were found. The regional hilar and bifurcational lymph nodes show little beyond mild sinus catarrh, but always contain much Pneumonyssus pigment.

The Pigment

The pigment, so characteristic of the lesions in monkey lungs, deserves particular reference, for it can be a diagnostic feature. Monkeys may acquire some anthracotic pigment, so all pigment present in any one monkey lung cannot be deduced to be of the Pneumonyssus variety. The latter has usually been considered to be the fecal excreta of the mite. Some histochemical tests have been made, but complete answers have not been achieved. Lillie[11] referred briefly to the difficulties attached to the differentiation of Pneumonyssus pigment from ordinary carbon particles. Histochemical tests applied to serial sections of pulmonary lesions gave the following results upon the pigment:

Test		*Result*
1. Schmorl's ferricyanide	(lipofuscin)	Positive
2. Nile blue sulfate	(lipofuscin)	Positive
3. Chrome hematoxylin	(lipofuscin)	Negative
4. Benzidine (hemoglobin)		Negative
5. Gmelin's (hematoidin)		Negative
6. Perls' (ferric)		Positive
7. Turnbull's blue (ferrous)		Positive
8. Cholesterol		Negative
9. Alizarin (calcium)		Negative
10. Hematoxylin lake		Positive
11. Tests excluding carbon, melanin, and formalin pigment		

There were two forms of pigment present in the monkey lungs. One was composed of golden brown, coarsely granular and needle-like crystals, free mostly in the perivascular lymphatic spaces away from the site of infestation. This material was anisotropic in crossed Nicol prisms, and was refractile in dark field illumination. All but the needle-like crystals (Fig. 12) gave a positive Schmorl's reaction. A smaller portion of the granules gave positive Perls' and Turnbull blue reactions for iron. Lipofuscin was present also. The other pigment was a finely granular material contained in phagocytes, or free in tissue spaces near and in the site of infestations. No needle-like crystals were apparent, and the pigment was more nearly homogeneous, was light

brown, refractile, and anisotropic. It was a strong reduction center for the Schmorl reaction and showed the presence of iron with the Perls and Turnbull blue tests. Lipofuscin was present. No hemoglobin was associated with the pigments.

It is certain that the pigment is derived from Pneumonyssus infestation in the monkey, since the presence of carbon and melanin was excluded by tests. Further, an examination of several lungs from other species of normal animals failed to reveal any refractile pigment. It is thus difficult to define the substance, but it is certainly iron-containing, and is probably derived from sucking and ingestion of monkey blood by the mite, and then breakdown and excretion of altered blood pigment. In both mites found within lesions, and in special preparations made of isolated parasites, by the hematoxylin and eosin stain, the same pigment was seen in internal structures, and hemoglobin was detectable as well as a positive inorganic iron reaction by special tests.

THE CAUSAL MITES (PNEUMONYSSUS SPP.)

It is unnecessary from the pathologic viewpoint to deal with the taxonomy of the causal mites. Full descriptions of Pneumonyssus were given by Ewing,[1] who gave other references including one to a standard text on Acarina by Banks.[12] There are several families of the order Acarina, of which one is Dermanyssidae to which the genus Pneumonyssus belongs. According to Ewing, six different species of lung mites from primates have been identified, but not all from Macaca. The more common have been *Pneumonyssus simicola* (Banks[12]), and *Pneumonyssus foxi* (Weidman, 1915) (see also paper by Vitzthum[13] and review by Oudemans[14]). Mites from our cases were identified as belonging to the former species on two separate occasions (1950 and 1953).*

With a little experience, mites (Figs. 4 and 5) can be isolated from virtually every pulmonary lesion visible to the naked eye. The focus can be squeezed between two glass slides, although it is better to tease it apart by needles, and then by using the dissection microscope, the movements of the mite can be used as a guide. The female measures about 0.75 mm. by 0.45 mm., and the male 0.55 mm. by 0.25 mm. (according to Ewing[1]), so they are within the range of macroscopic vision. One focus may contain only one, or as many as ten mites, and when first picked out they are covered by thick mucus. They will live

* In 1950 through the kindness of Dr. Edward W. Baker, Bureau of Entomology, U.S. Dept. of Agriculture, and in 1953 by Mr. Allen McIntosh, Zoological Division, Agriculture Research Center, Beltsville, Md.

for many days in saline solution or in media at room temperature, and for a few days in the refrigerator, but they rapidly become dry and die without moisture.

Discussion

Hitherto, records on pulmonary acariasis of monkeys have dealt with isolated sporadic cases, so that the literature might give the impression that the disease is of rare occurrence. If our observations are not unique, which seems probable, they give evidence that the disease is an enzootic one affecting, in some degree, most Macaca monkeys imported into the United States for experimental purposes and, perforce, other countries as well. If this is the case, there are grounds for a deliberate attempt to eradicate the disease by chemotherapy.

Many of our experimental monkeys are not necropsied for many months after importation and receipt into our normal stock colony. However, pulmonary lesions and the mites have been found in monkeys which have died within a few days of arrival, and in some killed on receipt within as little as 7 days after leaving India. It is certain that the animals acquire this infestation in their native country, India, Burma, or elsewhere in the Far East. Beyond that, there is little or no information regarding the life history of the mite and the pathogenesis of the disease. How, where, and when they acquire their original infestation can only be surmised. Nor do we know at what age they are first exposed and susceptible. We do not know how, or indeed, whether infection can be passed from monkey to monkey. Although in many lungs it might seem that all lesions by their size are at the same stage of development, it is difficult to believe there cannot be repeated reinfections. Monkeys supposedly could pick up these mites from grain or vegetation in their natural habitat, but the mites seem so susceptible to lack of moisture that it would be surprising if they were able to live apart from an animal host. We have not found Pneumonyssus on the skin of monkeys. Hence it is doubtful that they are ectoparasites which accidentally find their way into the lungs. Some workers have suggested that the ova are swallowed, and that after hatching the larvae pass via the lymph-vascular system into the lungs. This is unlikely, for the size of the larvae would prevent them from getting very far in the vascular system without causing infarction. A more plausible explanation would be that the mites crawl into and along the nasal cavities, and reach the lungs by direct wandering down the airway; mites have been observed in the air sinuses of monkeys by Oudemans.[14] All stages of the parasite have been found in the pulmonary lesions of monkeys, *viz.*, adult males and females, eggs, and

larvae. Consequently, the total life cycle might be passed within the lung. Cases showing multiple disseminated lesions may thus represent not only new exogenous infections by coughing and by nose and mouth contact from other affected monkeys, but also repeated endogenous reinfections by fresh hatchings, larval maturations, and new migrations.

Mite infestations of the lungs by *Pneumonyssus spp.* are stated to be found only in old-world monkeys (Ewing[1]), although Hamerton[6] referred to mite infestation in the lungs of one new-world monkey (Lagothrix). Ratcliffe,[15] pathologist at the Philadelphia Zoo, has also confirmed this, and has intimated that in his experience they occur only in genus Macaca. Lesions were seen by Ratcliffe in over 50 per cent of *Macaca mulatta* dying within 1 year after arrival, less commonly in older simian residents, only one of which had extensive and lethal lesions (a pigtailed *Macaca nemestina*). Rewell[16] recognized infestations of the lungs of monkeys by *P. simicola* and *P. foxi* in the London Zoo years ago, but only in few cases. He evinced surprise at the high incidence found by us in imported monkeys, but Macaca are not kept in zoos in large numbers, nor is the population transient, as in experimental laboratories. Zoos almost certainly receive animals of better quality in the first place.

Because of the very high incidence, it cannot be ignored that other animals (laboratory and domestic) kept on the same premises might become infested. We isolated adult mites, introduced them into the trachea of a rabbit, and found a few acari still alive in the lungs after 12 days, although whether true lesions, as seen in monkeys, were produced was questionable.

MacNamee, Yevich, and Bergner (1951–52) were able also to hatch ova, introduce the larvae into the lungs of rabbits and guinea-pigs, and recover living mites after 6 weeks. Experiments of this type are outside our province, and have not been extended further. They exemplify one important point, namely, that the parasites can survive in the lungs of abnormal hosts for some time, and that cross-infestation between monkeys and other mammals (even man) may be possible. This consideration was raised by Helwig,[2] Soysa and Jayawardena,[17] Rewell,[18] and by Davis,[19] and it is a trenchant point. As intimated by Soysa and Jayawardena, the finding of mites (not *Pneumonyssus spp.*, but Tyroglyphus and Tarsonema) in human sputum suggested possible causes for certain tropical pulmonary disorders such as asthma, bronchitis, and Loeffler's syndrome, the cause of the latter being still unknown. The simian disease offers a means whereby pulmonary

acariasis can be studied further under natural conditions; and, if very young monkeys are found free from infestation and are available in sufficient numbers, under strict experimental control and in natural hosts.

The lesion is a peculiar one, partly because of its discrete restriction to the small pulmonary area where the mites lodge. It is not a granuloma, nor bronchopneumonia, nor like a tubercle, and in most cases is not a parasitic cyst in the ordinary sense. Most of the lesions are a localized bronchiolitis and peribronchiolitis with some degree of ectasia. This affects only a short segment of the tube wherein the mite resides, and does not extend for a great distance along the bronchioles except when the mites migrate. To a lesser extent there are other lesions of focal lobular pneumonitis. From this and other evidence, it may be that the mites travel only along the paths of least resistance, namely, the lumina of the bronchial channels, until perhaps the size of the lumen prevents further progress. The mites may not actually burrow hither and thither in lung tissue, as happens in the vicious dermal wanderings of Sarcoptes and Demodex mites which cause mange in animals; those mites have been found to migrate even to the lymph nodes in severe cases of demodectic mange in dogs (Lucker and Sause[20]).

The focus around the mite ensues as a specific cellular reaction, and cannot be confused histologically with any other process, but macroscopically the lesions may have been mistaken repeatedly for tuberculosis. It is apparent from a re-examination of slides from cases of monkey tuberculosis, seen by one of us (J. R. M. I.) many years ago, that mite lesions were present along with those due to the tuberculous infection.

It might be assumed that the lesions are produced by simple mechanical trauma and irritation due to the mites; however, all pulmonary endoparasites do not produce the same pathologic picture, and mite lesions are specific and not like those caused by physical irritants. In sheep and goats, pulmonary disease caused by various helminths may be subacute or chronic, or may spread widely through lungs and kill the animal by a lobar pneumonia. The curious marked proliferation of plain muscle fibers, present in certain chronic parasitic pneumonias of sheep, goats, and deer, has not been observed in the mite disease, although a slight degree was seen in some affected bronchiolar walls. Mechanical irritation may not suffice, therefore, as a simple explanation of the genesis of the pulmonary acariasis. In the mite lesion there is no similarity to the chronic condition of pulmonary adenomatosis, seen classically in jaagsiekte of sheep, stated to be caused by a virus.

It is unknown whether the process is self-limited, in that the parasites might die if the monkeys live long enough, and the lesions might resolve. If this were so, multiple fibrous scars might be expected in monkey lungs. Such scars with dimpling of the pleural surface have almost never been noticed by us.

The pigments present in the lungs, often in large amounts, and not necessarily in direct spatial association with mite lesions, are interesting and comments have been made on their histochemical nature. As anthracosis must be rare in monkey lungs, these pigments could be a diagnostic feature of mite infestations. As there is rarely any hemorrhage in or near mites, it would seem that the acari attach themselves to bronchiolar walls, and literally live as permanent blood-suckers, excreting broken-down blood pigment.

It is difficult to see a solution to control of the disease, although this is a prime consideration. One important feature is that the disease is clearly enzootic, although of low grade pathogenicity. It thus becomes a potential complication in any experimental work on monkeys with specific reference to pulmonary pathology. Neither clinical signs, roentgenologic examinations, nor hematologic tests (presence of eosinophilia) are of diagnostic value in the living animal. Yet, Carter, Wedd, and D'Abrera,[9] in Ceylon, in human cases with a chronic respiratory disorder in which they found mites in the sputum of 17 of 28 persons, observed an eosinophilia ranging from 38 to 68 per cent. Soysa and Jayawardena,[17] in their 30 human cases, found an eosinophilia ranging from 33 to 81 per cent, and in one case there was a total count of 27,000 eosinophils per cmm. In the cases of Carter *et al.*,[9] the eosinophilia was greatly reduced, the respiratory condition greatly improved, and the mites expectorated by treatment with pentavalent arsenicals (stovarsol was used). This medicinal treatment might possibly be tried therapeutically in imported monkeys, and experiments along this line are in progress.

Summary

In over 400 post-mortem examinations of imported old-world rhesus monkeys made in these laboratories, lung mites (*Pneumonyssus spp.*) and their lesions were an almost universal finding. The disease is enzootic in such imported animals, and it might be a complicating factor which could influence interpretation of experimental work, especially that involving the lung. The disease cannot be diagnosed clinically nor roentgenologically, nor is there any constant associated eosinophilia of the peripheral blood. The lesions form scattered discrete foci in the lungs, varying in severity and dissemination in individual monkeys. The process is characterized by the production of a localized bronchiolitis and peribronchiolitis, or focal pneumonitis in

which eosinophils may be prominent. The focus rarely becomes lobar, but there may be some degree of bronchiolectasis. Pigments and doubly refractive crystals are constantly present in, near, and distant from lesions or from mites. At least a part of the pigment and crystals is derived from mites sucking and ingesting blood from the host, and excreting breakdown products of hemoglobin. Little is known regarding the pathogenesis. Reference is made to the findings of mites in sputum from human patients with respiratory disorders in Ceylon and India by other workers, and to the suggestion that mites may be a cause of human pulmonary disorders occurring in the tropics, including asthma, bronchitis, and Loeffler's eosinophilic lung lesions.

Our thanks are due to Mr. John Cuculis, Pathology Branch, Medical Laboratories, who has been responsible for all the photographic work.

ADDENDUM

Since this paper was accepted for publication, much personal discussion (and correspondence) has been had with workers in the United States and elsewhere who have been interested in the problem. One group (Drs. Lee, Williams, Hull, and Stein, Naval Medical Research Institute, Bethesda, Maryland), made a study of 26 cases and found some evidence of an associated elevated sedimentation rate, hypoproteinemia, and eosinophilia. They also tried therapeutic treatment with cortisone and pentavalent arsenicals. A brief account of their work was presented to the Federation of American Societies for Experimental Biology, April 16, 1954. (Lee, R. E., Williams, R. B., Jr., Hull, W. B., and Stein, S. N. Significance of pulmonary acariasis in rhesus monkeys (*Macaca mulatta*). *Federation Proc.*, 1954, 13, 85–86.)

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[*Illustrations follow*]

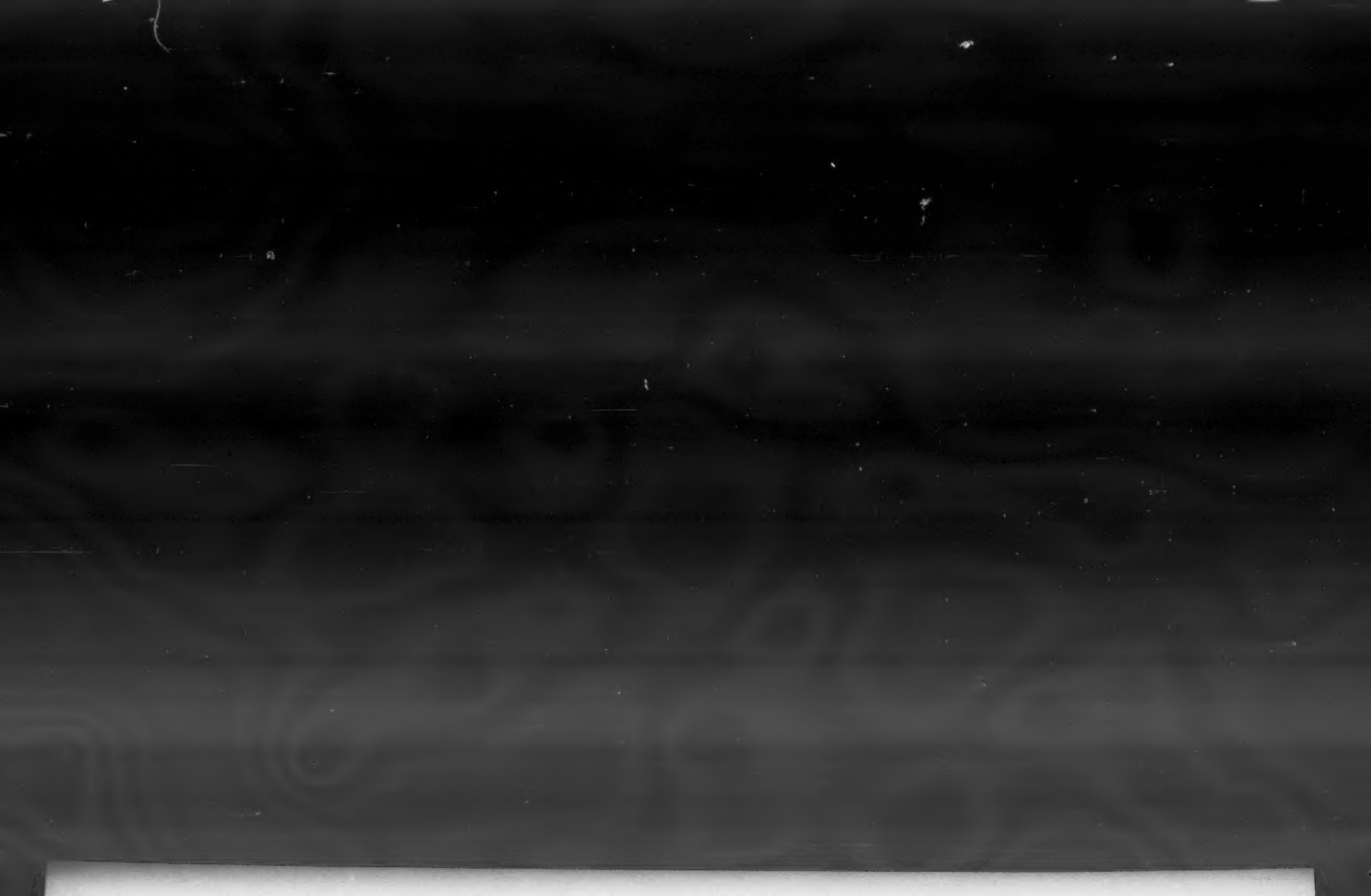
LEGENDS FOR FIGURES

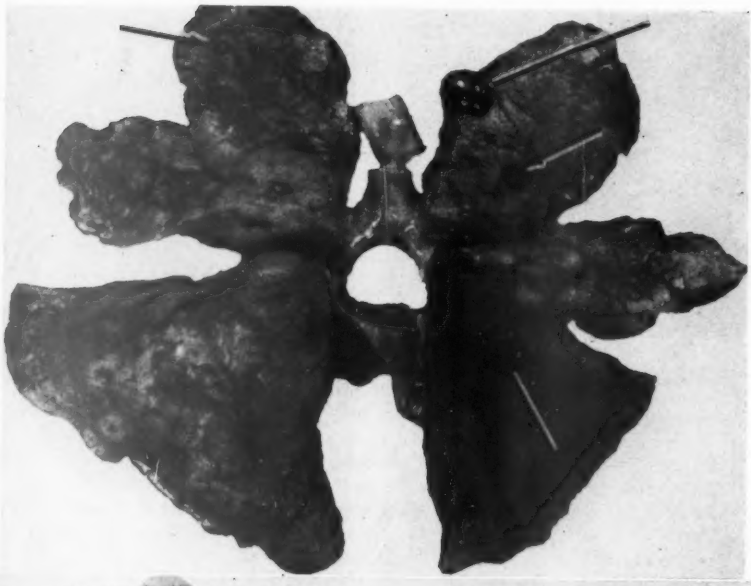
FIG. 1. Pulmonary acariasis, monkey. Macroscopic appearances. Typical common, discrete, focal lesions are marked by arrows, and lesions of the more rare bullous type, projecting well above the pleural surface, are marked with a rule.

FIG. 2. Pulmonary acariasis, monkey. Lungs. Another case showing focal subpleural lesions larger than those in Figure 1.

FIG. 3. Pulmonary acariasis, monkey. Lungs. Appearance of a pulmonary lesion teased and examined under the dissection microscope; five small hillocks of the mites embedded in mucus (arrows) can be distinguished.

FIGS. 4 and 5. Mites *(Pneumonyssus simicola)*, pulmonary acariasis, monkey. Figure 4, larva with six legs, and Figure 5, adult female taken from a lung focus. Unstained and partly squeezed. Approximately × 50.





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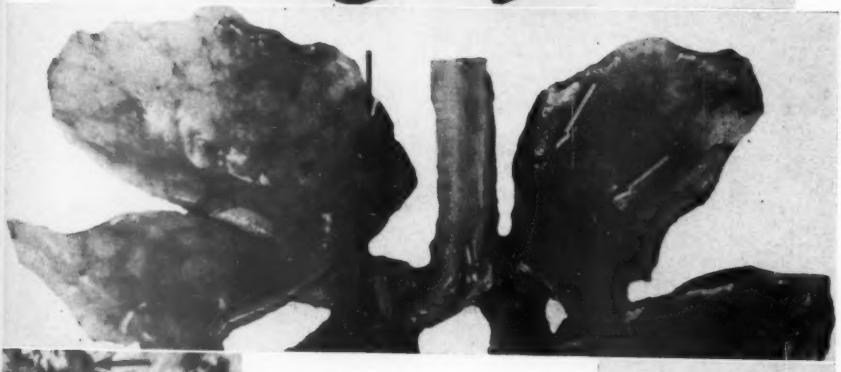

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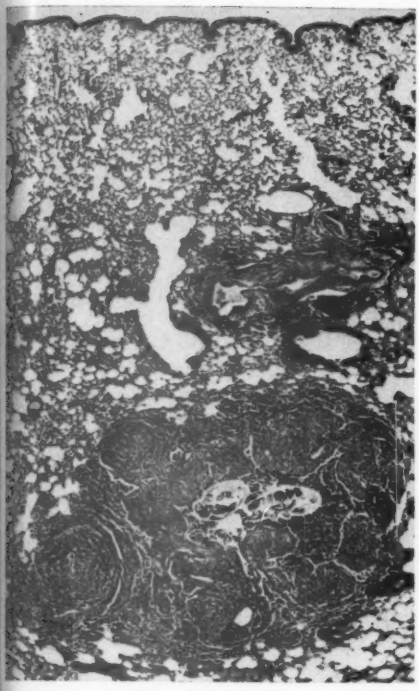
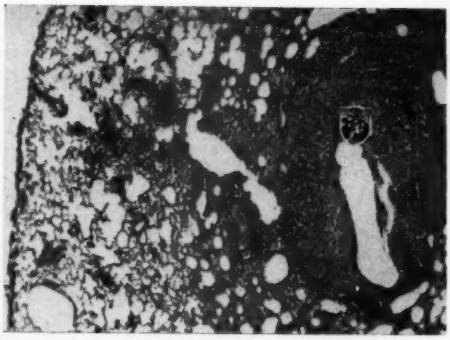
FIGS. 6 to 9. Pulmonary acariasis, monkey. Lungs. A series of lesions from different cases, demonstrating variations in the histologic picture. Figures 6, 7, and 8 illustrate the standard pattern affecting bronchioles with central irregular lumina, destruction of epithelium and parts of mucosa, and thickening of the walls by cellular infiltration; pigment can be seen also; alveoli adjacent to bronchiolar lesion show some collapse; proximity to pleura can be seen in Figures 6 and 7; parts of mites can be seen in Figures 6 to 9. Figure 8 shows irregular ectasia. Figure 9 shows infrequent type of thin-walled dilated bronchiole (cyst) empty of detritus or mucus, segments cut at different levels, with parts of larva near wall. Prominent lymphoid follicles are shown in Figure 6.





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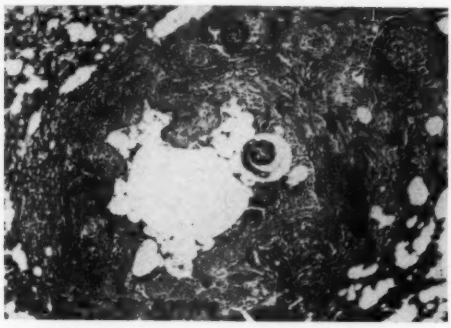

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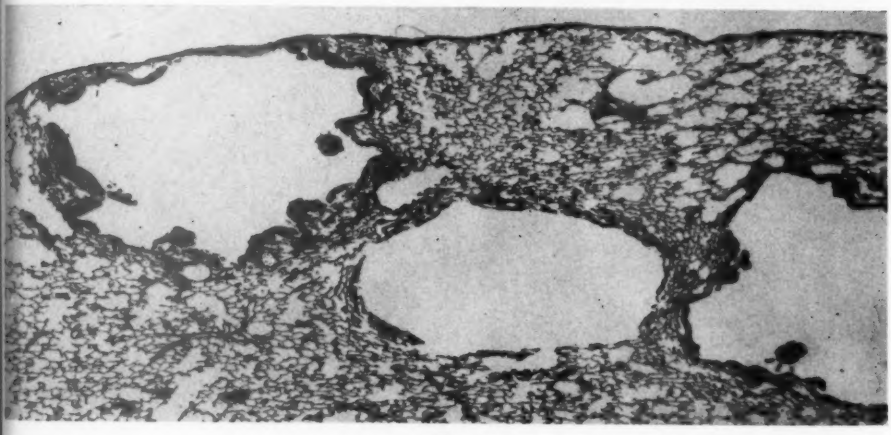

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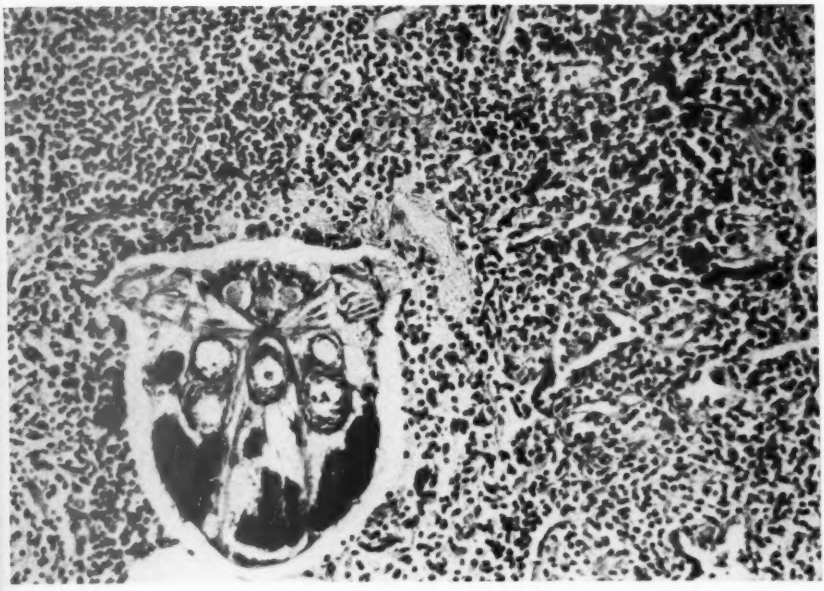
FIG. 10. Pulmonary acariasis, monkey. Lung. Portion of bronchiole from Figure 7, with thickened infiltrated wall and massive pigment deposition. A section of the mite is included. Hematoxylin and eosin stain. × 150.

FIG. 11. Pulmonary acariasis, monkey. Lung. Part of affected bronchiolar wall showing peculiar proliferation of plain muscle. Hematoxylin and eosin stain. × 150.



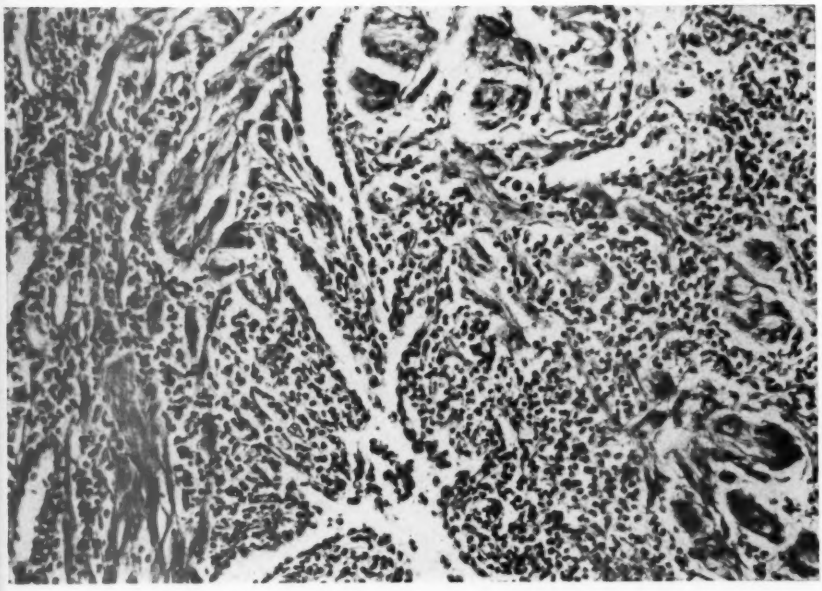


FIG. 12. Pulmonary acariasis, monkey. Lung. Perivascular granular pigment and needle-shaped crystals from an area not immediately adjacent to a mite lesion. Hematoxylin and eosin stain. × 900.

FIG. 13. Pulmonary acariasis, monkey. Lung. A focus of pneumonitis containing pigment and almost certainly caused by a mite. This is the only lesion present in the section, and illustrates a microscopic finding which may be found incidentally in routine examinations of simian lungs. Hematoxylin and eosin stain. × 100.

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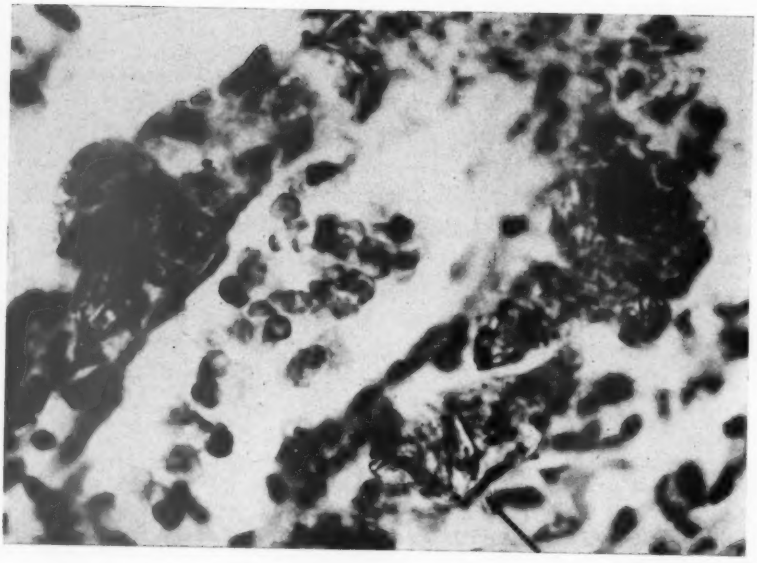

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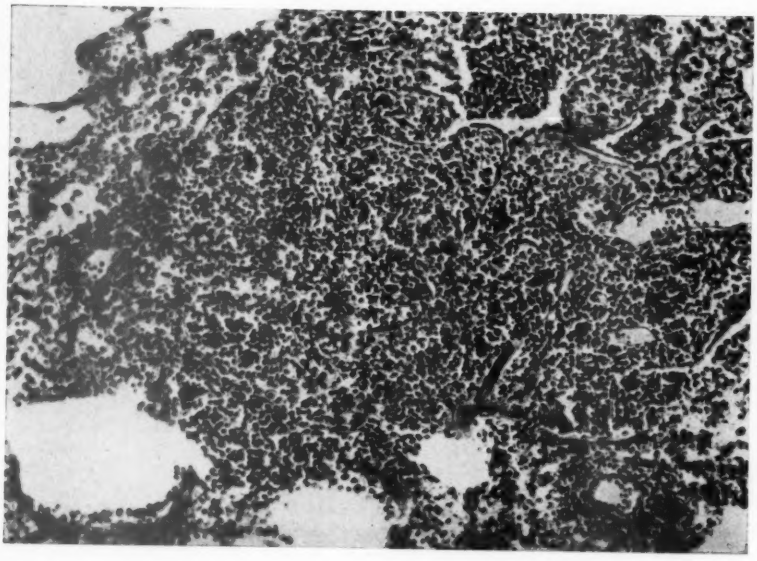

THE COMPARATIVE PATHOLOGY OF RHABDOMYOSARCOMA WITH A REPORT OF A CASE IN A DOG *

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Malignant tumors of striated muscle origin have been reported from animals in only a few instances. A review of the available literature does not reveal a definite report of a neoplasm of this type in a dog. Rudduck and Willis,[1] however, described a "spindle cell sarcoma (possibly rhabdomyosarcoma) of the heart" of a dog. Although many iron-hematoxylin sections were examined, cross striations were not observed. In view of the paucity of reports, we believed that presentation of the following case of a primary metastasizing neoplasm of striated muscle in a dog is indicated.

REPORT OF CASE ‡

An 8-year-old male English setter was admitted to a local veterinary hospital with the owner supplying a history of loss of weight and lameness of the left rear leg. On examination a firm, irregular swelling was palpated on the medial aspect of the left stifle joint. The swelling appeared to lie in the subcutaneous tissue between the skin and the striated muscle and measured approximately 9 cm. in its greatest dimension. The animal succumbed the following day.

POST-MORTEM EXAMINATION

At necropsy, the outstanding finding was the presence of multiple nodular metastases in the lungs, liver, spleen, kidneys, mediastinal lymph nodes, and adrenal glands. The involvement was most marked in the lungs and liver. The larger nodules attained a diameter of approximately 5 to 6 cm., and the smallest was pin-point in size. These lesions were white and firm.

Primary Lesion. The specimen from the leg was an irregular nodular mass measuring 7 by 4 by 4 cm. The external surface (Fig. 1) had a fibrous capsule along one margin and irregular strands of striated muscle and connective tissue along the opposite surface. Irregular nodules of varying size projected from beneath the capsule. On cut section (Fig. 2) an ill defined fibrous capsule surrounded the greater portion of the tumor. There were multiple nodules of fairly firm neoplastic tissue which had a cellular gray appearance with foci of light

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‡ We are indebted to Dr. A. M. McDermid for supplying the clinical history and necropsy report.

yellow pigmentation. These nodules varied from 0.3 to 1.5 cm. in diameter. The central portion of the entire mass was hemorrhagic, necrotic, and showed areas of mucinous consistency.

Lungs. The lungs showed numerous gray tumor nodules beneath the pleura (Fig. 3). These varied in diameter from 0.3 to approximately 4.0 cm., and some of them projected above the pleural surface. On cut section (Fig. 4) the nodules were found to be cellular and rather rubbery. There was a grayish mottled appearance with a suggestion of hemorrhagic foci.

Liver. There was a large nodule projecting from the liver surface (Fig. 5). It measured approximately 4.5 cm. across, had a gray appearance like that of the pulmonary lesions, and was of rubbery consistency. On cut section foci of hemorrhage were evident and there was compression of the adjacent hepatic parenchyma.

Adrenal Gland. The adrenal gland examined (Fig. 6) measured about 4 cm. in its greatest dimension, and had two hemorrhagic nodules in the medulla which were believed to be metastases. The larger measured approximately 1.5 cm.

Microscopic Examination

Primary Lesion. Sections of the primary lesion revealed a poorly defined, irregular, fibrous and striated muscular pseudocapsule, infiltrated with inflammatory cells; beneath and extending through this pseudocapsule were nodules of the neoplasm (Fig. 7). The individual nodules were separated by a fairly dense, well vascularized connective tissue together with bundles of striated muscle which showed atrophy and some fibrosis. There was considerable variation among the tumor cells. Many were polyhedral with nuclei of varying shapes, the dominant nuclear form being oval. Present in large numbers were cells of another type, somewhat elongated with spindle-shaped nuclei. There were occasional multinucleated tumor giant cells (Fig. 10), which were usually oval and some of them contained as many as twelve nuclei. The cytoplasm of these giant cells, as well as of the other tumor cells previously described, was eosinophilic (hematoxylin and eosin preparation). The cytoplasm of the giant cells was granular, but this was not observed in cells of the other types. Within the primary neoplasm tumor cells with definite striations were not demonstrated. There were areas of degeneration which varied from edema to necrosis; some of these areas exhibited marked hemorrhage. Scattered deposits of iron pigment were present in phagocytic cells, and a small quantity of free pigment was found.

Lung. Sections through the metastatic areas within the lung (Fig. 8)

also exhibited a varied histologic pattern, the variation being more marked than in the primary growth. Similar types of cells were encountered, but there were many more elongated strap or ribbon cells, some of which were multinucleated. The nuclei of these elongated cells were peripherally located. These elongated cells varied in contour, some being relatively linear, still others curved and S-shaped. They varied as much as four to five times in width from the smallest to the largest. A few of these strap-like cells showed definitive cross striations (Fig. 11). Another type of multinucleated cell was larger and somewhat oval, with a granular eosinophilic cytoplasm (Fig. 9). These cells were similar to the giant cells of the primary neoplasm; they were, however, encountered more frequently in the metastatic growth. The lung metastases contained areas of necrosis. A considerable quantity of edema fluid was present within adjacent alveoli; still other areas exhibited atelectasis near the nodules.

Liver. The section of the metastatic growth in the liver had a pattern somewhat similar to that encountered in the lung, although the degree of cellular pleomorphism was not so marked. Definite cross striations were not found in these tumor cells, even though their morphologic features were very similar to those of the cells in the pulmonary metastases. The adjacent hepatic parenchyma had marked sinusoidal dilatation. There was marked atrophy of the hepatic cord cells, with distortion of the normal architecture.

Adrenal Gland. The medulla contained metastases which projected into the cortex. There were areas of hemorrhage and edema.

Review of Literature

According to the available literature, reports of striated muscle tumors of animals are rather few. Boucek[2] described a rounded mass in the myocardium of a cow. Because of the presence of glandular elements, the tumor was classified as an adenorhabdomyoma. In addition, Hieronymi and Kukla[3] reported multiple rhabdomyomata of the heart of a 4-month-old pig.

Day[4] reported the finding of a rhabdomyoma in a lamb. When the carcass was opened at the packing plant, both lungs showed numerous spherical nodules of varying size; and a diagnosis of "rhabdomyoma" was made by microscopic examination. Even though the inspector at the plant reported that all the other organs were normal in appearance, the nodular distribution of the tumor in the lungs, together with the definite cross striations evident in the published photomicrograph, suggest that the neoplasm was metastatic. From the text and from the photographs, the possibilities that this was a metastatic rhabdomyo-

sarcoma, or a metastatic Wilms' tumor of renal origin (particularly since there were described in the pulmonary lesions tubular structures with epithelial cells resembling those "lining the tubules of the kidney") cannot be excluded. Day's interpretation was that the neoplasm arose from misplaced embryonal tissue and that the glandular elements represented remnants of the wolffian body whereas the striated muscle cells arose from myotome inclusions.

Rhabdomyomatous tumors have been observed in the skeletal muscle of fish. Reference in Nicory's paper[5] was made to two previous reports of these tumors; one in a codfish and the other in a lake trout. Kolmer[6] reported finding a rhabdomyoma in a tench, while Haddow[7] referred to a previous report of a rhabdomyoma of a turbot.

Feldman,[8] in his book on *Neoplasms of Domesticated Animals,* stated that he was able to find mention of "less than twelve cases" which he considered to be tumors of striated muscle origin, and he made reference to several reports which are reviewed here. As to some of the cases mentioned in Feldman's review, one is not entirely convinced of the origin of the tumor from striated muscle. In addition, some mixed tumors were included in this brief series. Feldman referred to a rhabdomyoblastoma in a 13-year-old horse (case report of Coyne and Cavalié[9]) which measured 6 by 5 cm., had a red brown color, and was located under the skin in the region of the shoulder. In addition, Feldman mentioned Joest's case[10] of a tumor in the heart of a hog and also Prochazka's report[11] of a rhabdomyoblastoma in the neck of a 6-month-old bovine fetus. In his series of animal neoplasms, Feldman described two rhabdomyoblastomas. One of these was from a 5-year-old gelding and was located over the right femur while the second specimen was in the prescapular region of a 1-year-old sheep.

Jackson,[12] in his study of the incidence and pathology of tumors of domesticated animals, reported no striated muscle neoplasms.

Rudduck and Willis[1] described a tumor of the heart of a dog with multiple metastases to the lungs and a single metastatic diaphragmatic nodule. Histologically, the tumor consisted of two cell types, spindle and pleomorphic. Because of the appearance of the spindle cells, these authors considered the neoplasm as probably being a rhabdomyosarcoma; they were not, however, able to demonstrate definite cross striations.

One case in a guinea-pig has been reported; Hueper[13] observed in the heart a rhabdomyoma measuring 0.5 by 0.75 cm. He considered that the tumor reported by him was a congenital tissue malformation and not a true neoplasm; a point of view held by most pathologists with reference to the so-called rhabdomyomas of the heart.

Mulligan,[14] in his introductory notes to *Neoplasms of the Dog,* mentioned one case of rhabdomyosarcoma in his classification of 1,000 neoplasms. He did not include a description of the tumor, but mentioned in a later chapter that a rhabdomyosarcoma occurred along the lateral border of the tongue; this was associated with a carcinoma of the minor salivary glands at the base of the tongue.*

Recently, Krogh[15] in Sweden reported two cases of rhabdomyosarcoma in White Leghorn chickens.

Discussion

Malignant tumors of striated muscle origin are infrequent in man, and Willis[16] commented: "relatively rarely is the adult skeletal musculature of the body clearly and unmistakably the origin of a rhabdomyosarcoma." He commented further that "most rhabdomyomatous tumours are either embryonic growths derived from immature myoblastic or undifferentiated mesenchymal tissue, or mixed tumours in which other heterotopic tissues beside muscle are differentiated."

In lower animals only a few descriptions of striated muscle tumors are recorded, and Feldman[8] pointed out that they are so rarely observed that "opportunity for their comprehensive study has not occurred." The case reported in this article is the only example which we have been able to find of an established primary neoplasm of striated muscle in lower animals with metastases to distant organs.

In the differential diagnosis of the primary neoplasm in this case, one must give consideration to a rhabdomyosarcoma and to a fibrosarcoma. The finding, however, of cross striations in the metastatic lesions in the lungs provides proof, we believe, of the striated muscle origin of the neoplasm. Probably some neoplasms of the deeper tissues are in reality of striated muscle origin, but are classified as fibrosarcomas in view of a lack of cross striations, or a failure to demonstrate them.

It is of interest that the greatest cellular variation in the neoplasm occurred in the pulmonary nodules, and speculation arises as to whether the neoplasm may grow more freely in an area in which pressure effects of adjacent tissue may be less marked. The neoplasm contained more differentiated cells in the lungs than elsewhere. This observation is in agreement with the findings of Burgess[17] who noticed the greatest degree of tumor cell differentiation in the lungs and peribronchial lymph nodes of a 19-year-old girl who had a neoplasm of

* A personal communication from Dr. Mulligan indicates that special staining procedures have led to a reclassification of the neoplasm and that this case is now considered a hemangiopericytoma.

the extensor muscles of the thigh with numerous distant metastases.

Considerable discussion has been evoked in the past relative to whether rhabdomyomatous growths arise from already mature striated muscle fibers or whether they arise from immature myoblastic elements and are embryonic growths. We can make no definite comment relative to this, although we are of the opinion that the tumor presented here arose from the striated muscle cells in the region of the stifle joint of this 8-year-old dog.

In regard to the point of origin of the neoplasm reported by Burgess[17] which has been referred to, he commented: "The fact that the largest nodule was situated in the extensor muscles of the thigh renders it not improbable that it was from the cells of those muscles that the new growth arose." A similar point of view is shared by us in regard to our case.

Willis[16] mentioned that nearly all unquestionable striated muscle growths arise early in life, with the exception of the mixed tumors of the uterus. However, the striated muscle tumor which is reported in this article occurred in a dog of 8 years and this would be considered as in the older age group for dogs.

Summary

A primary neoplasm of the striated muscle of the thigh of an 8-year-old dog is reported. Metastatic nodules were observed in the lungs, liver, spleen, kidneys, mediastinal lymph nodes, and adrenal glands. Definite cross striations were found only in the lung. The rarity of this neoplasm in the field of comparative pathology is evident from a review of the pertinent literature.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Posterior view of specimen from medial aspect of the left stifle joint.

FIG. 2. Cut section of the specimen from the leg.

FIG. 3. Lung tissue with subpleural nodules.

FIG. 4. Cut section of lung with metastatic nodules.

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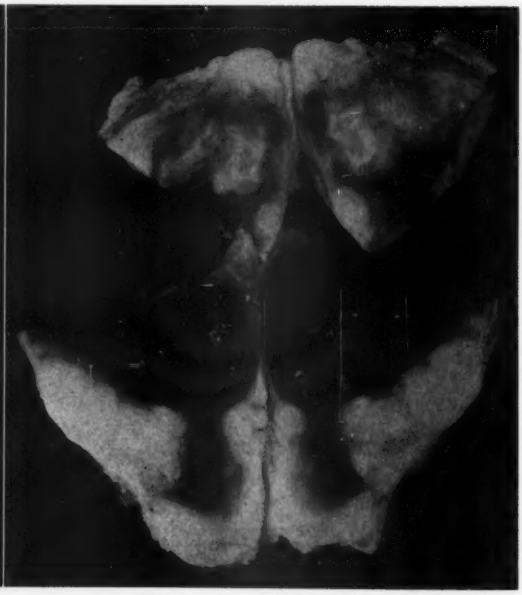

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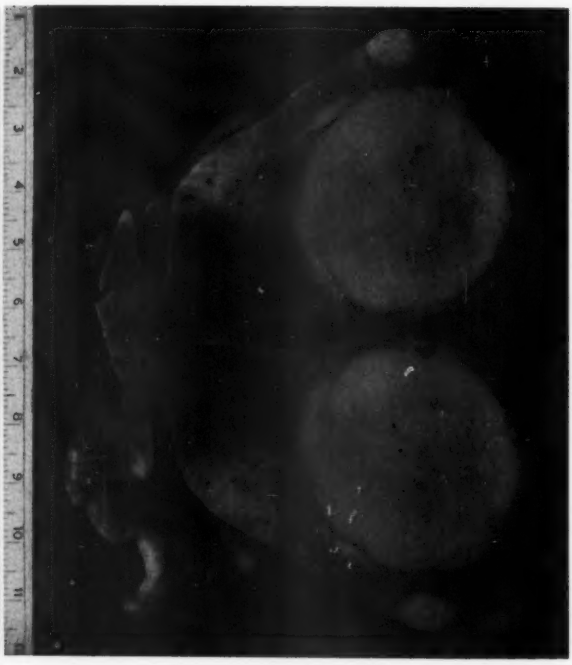

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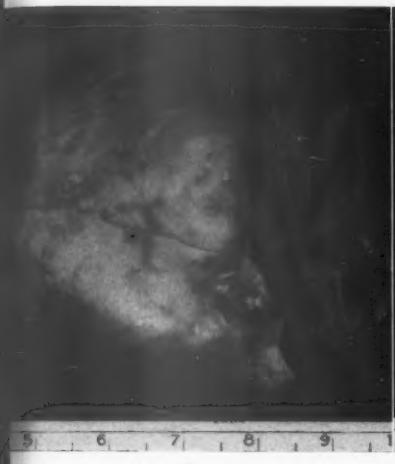
FIG. 5. Large nodule projecting from liver surface.

FIG. 6. Adrenal gland with metastatic areas.

All sections were prepared from formaldehyde-fixed tissue. Hematoxylin and eosin stains were used for the sections in the figures.

FIG. 7. Tissue from the primary tumor of the leg. × 75.

FIG. 8. Metastatic neoplasm in the lung. × 75.



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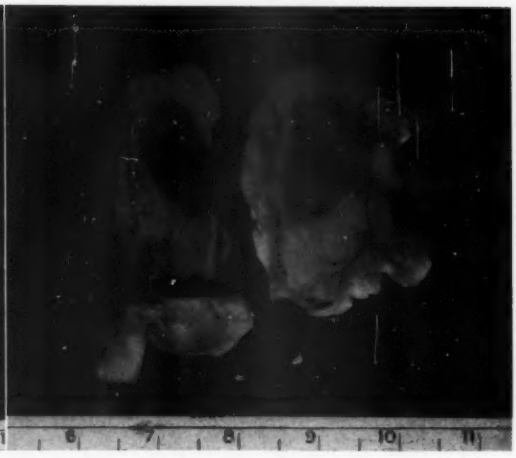

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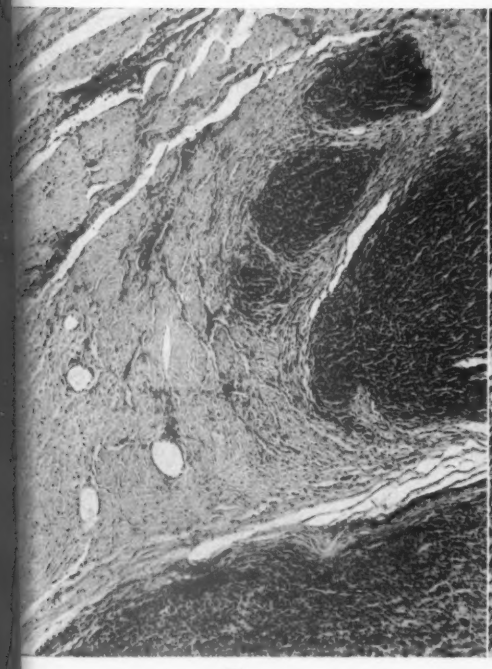

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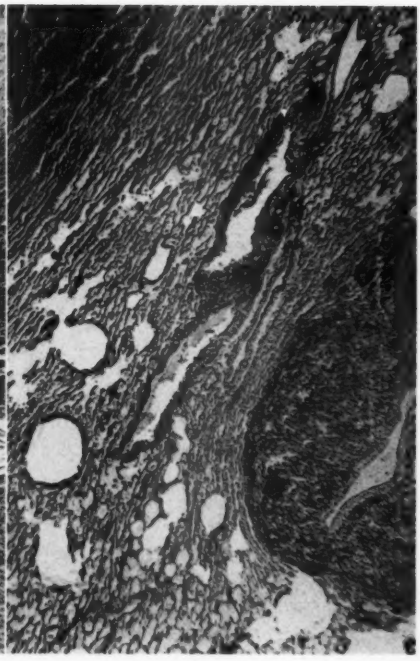

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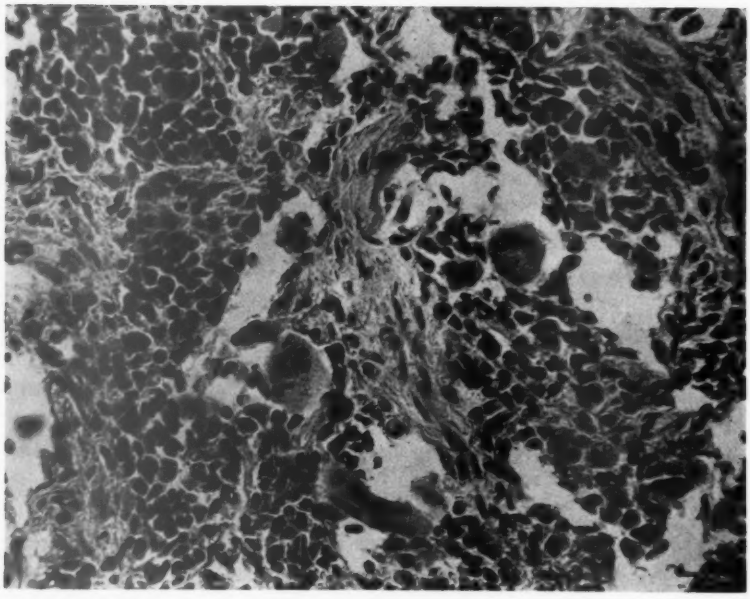
FIG. 9. Neoplastic tissue of the lung with varied cytologic pattern. × 300.

FIG. 10. Giant cells in the primary tumor. × 275.

FIG. 11. Striated muscle cell in a metastatic nodule in the lung. × 1008.



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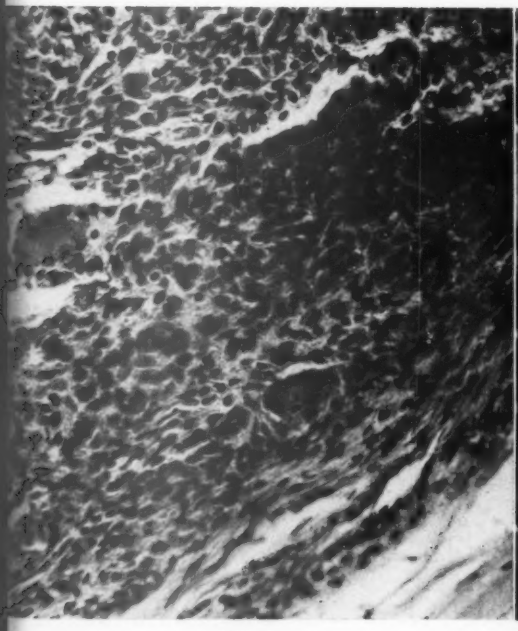

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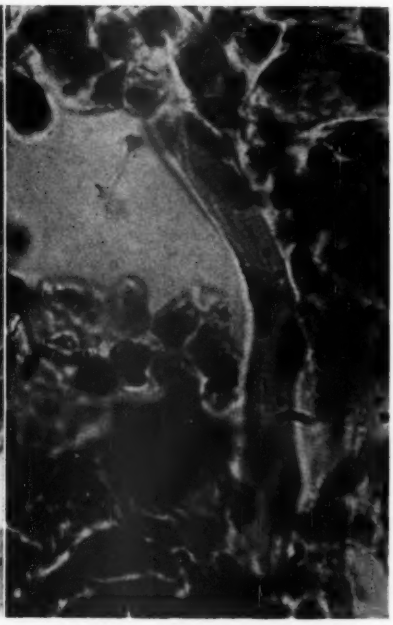

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THE OCCURRENCE OF ARTERIOSCLEROSIS IN THE AORTA OF SWINE *

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Intensive searches in the medical and veterinary literature have revealed that there is a paucity of factual information concerning the incidence of arteriosclerosis in swine. The spontaneous occurrence of arteriosclerosis in that species has been mentioned only incidentally by Fox,[1] Creech,[2] and Anthony.[3] The impression these authors convey is that the condition is exceedingly uncommon and so seldom observed that descriptions are lacking in detail. Apparently, investigative studies have not been made of the spontaneous rate of such lesions and their morphology in the swine. In connection with other studies on comparative arteriosclerosis, it was deemed advisable to obtain quantitative data upon the spontaneous occurrence of arteriosclerosis in the porcine aorta.

MATERIALS AND METHODS

More than 2,000 aortas were examined in fresh condition at a local and two Chicago slaughterhouses. The animals from which the aortas were obtained ranged in age from about 4 months to more than 3 years. The pigs were gilts and shoats, while breeding sows, boars, and stags made up the older groups. Representative lesions from the arch, the thoracic, and the abdominal aorta from different age groups were fixed in 10 per cent formalin. The tissues were embedded in paraffin, and then sectioned and stained with hematoxylin and eosin for microscopic study.

RESULTS

Gross Examination

The gross appearance of the arteriosclerotic lesions varied, as can be seen in Figures 1 and 2. The minimal visible lesions appeared as small (0.5 to 1.5 mm.), pale yellow, elongated plaques, hard to the touch under the endothelium, or as larger (5 to 10 mm.), thickened portions of the intima. The small lesions appeared to be aligned nearly parallel to the long axis of the aorta. The greater numbers of plaques were seen in the descending arch and upper thoracic region. One old boar showed over 60 individual minimal plaques in these regions.

The relation between age and the occurrence of arteriosclerotic lesions is apparent from Table I. The incidence was 3 per cent in the

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pigs and showed a progressive tenfold increase in the oldest groups. There were no marked differences in the sex incidence of the same age groups. Arteriosclerosis of the abdominal aorta in swine appeared to be relatively less frequent. Of 50 sows, 2 to 3 years old, 14 had plaques in the arch and thoracic aortas, whereas only one had sclerotic

TABLE I

The Relations Between Age and Sex of Swine at Slaughter and the Incidence of Aortic Arteriosclerosis

Age	Sex	Aortas examined	Sclerotic aortas	
years		*no.*	*no.*	*per cent*
⅛–⅜	F and M	493	16	3
1–2	F	676	91	13
2–3	F	362	92	25
2–3	M	28	7	25
3+	F	69	21	30
3+	M	147	51	35

changes in the abdominal aorta. The intima was depressed and puckered rather than elevated and irregular in outline. In two other sows these 1 to 2 mm. depressions in the abdominal aorta measured from 4 to 5 mm. across, and they were a faint reddish brown.

Microscopic Examination

The plaques in the arch and thoracic aorta were intimal elevations which were principally composed of fibrous connective tissue. Cholesterol clefts, focal hemorrhages, or phagocytes containing lipid vacuoles were not observed in these plaques. However, minute quantities of sudanophilic material have been observed in and around the fibroblasts in most of them. The sections taken from the discolored abdominal aorta were different. There were foci of minimal edema, hyalinization and disruption of the elastic connective tissue fibers, and atrophy of the media. In or around such areas irregular basophilic granules, presumably calcium precipitate, were encountered.

SUMMARY

Fresh aortas were examined from more than 2,000 swine which varied in age from 4 months to more than 3 years. The incidence of arteriosclerotic plaques was found to increase with age. Firm white or pale yellow intimal plaques of 1 to 10 mm. in size were encountered principally in the descending arch and the thoracic aorta. Such plaques accounted for almost all of the lesions observed, and they were com-

posed of fibrous connective tissue. Sclerosis of the abdominal aorta is more rare. In two of three instances when such lesions were encountered, calcium precipitate was observed in the media.

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[*Illustrations follow*]

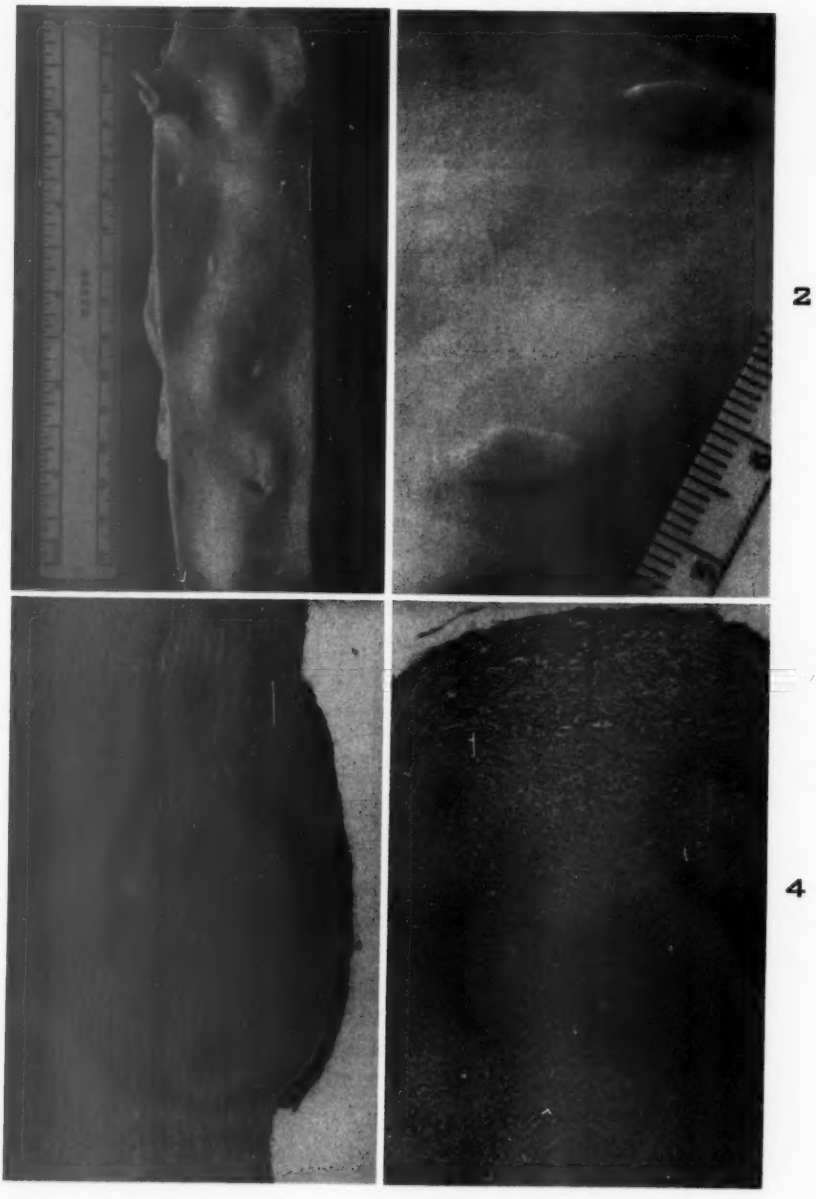
LEGENDS FOR FIGURES

FIG. 1. Photograph of a fresh aorta from a 3-year-old hog. The firm, flat (0.5 to 3.0 mm.) plaques are visible in the thoracic aorta. There is parallel alignment along the long axis of the aorta.

FIG. 2. Photograph of an aorta from a 3-year-old hog, at greater magnification. The scale at the lower right is in millimeters. The periphery is irregular but well defined. The intervening intima is smooth.

FIG. 3. The intimal plaque is composed principally of fibrous connective tissue. There is an increase of hyaline material at the base of the plaque. The surface of the nodule is smooth. Hematoxylin and eosin stain. × 150.

FIG. 4. This intimal plaque is larger and the hyalinization is more extensive than in the plaque shown in Figure 3. In addition to the intimal change, there is hyalinization and disruption of elastic fibers, and calcium precipitation in the inner media. Hematoxylin and eosin stain. × 150.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